Proceedings
of the
Society
for
Experimental Biology and Medicine

VOL. 48. DECEMBER, 1941. NO. 3.

SECTION MEETINGS

CLEVELAND	
Western Reserve University	November 14, 1941
IOWA	
State University of Iowa	November 19, 1941
MINNESOTA	
University of Minnesota	November 19, 1941
NEW YORK	
Cornell University Medical College	November 12, 1941
ROCKY MOUNTAIN	
University of Utah	November 1, 1941
SOUTHERN	
Tulane University	November 28, 1941
SOUTHERN CALIFORNIA	
University of Southern California	October 23, 1941

13384

Inadequacy for Mice of a Synthetic Diet Supplemented with All Known Vitamin B Factors.*

ELIZABETH TROESCHER-ELAM AND HERBERT M. EVANS.

From the Institute of Experimental Biology, University of California, Berkeley.

Since the work of Beard[1] and of Bing and Mendel,[2] the mouse has until recently been largely neglected as a subject for research on

---

* Aided by grants from the Research Board and the Department of Agriculture of the University of California, the Rockefeller Foundation, and Merck and Co., Rahway, New Jersey. Assistance was rendered by the Works Project Administration, Official Project No. 65-1-08-62, Unit 5-A.

[1] Beard, H. D., *Am. J. Physiology*, 1926, **75**, 645.

[2] Bing, F. C., and Mendel, L. B., *J. Nutrition*, 1929, **2**, 49.

the vitamin B complex. A great interest in this animal has arisen suddenly in several quarters.

Norris and Hauschildt[3] reported that the "filtrate factor" would not cure the skin symptoms exhibited by mice receiving pyridoxin, thiamin, nicotinic acid and riboflavin as factors of the B complex. Sandza and Cerecedo[4] and Martin,[5] however, found that these symptoms could be cured by pantothenic acid, a most important constituent of the "filtrate factor." The series of experiments carried out by Woolley[6-9] have demonstrated the importance of inositol, in addition to pantothenic acid, for the growth and hair maintenance of mice, although Sandza and Cerecedo[4] and Martin[5] have been unable to demonstrate that lack of inositol produces an alopecia.

Ansbacher[10] has claimed that p-aminobenzoic acid is a chromotrichia factor for mice as well as for rats.

According to Carruthers,[11] synthetic diets containing a high percent of yeast as a source of the vitamin B complex, are inadequate for mice; fresh flaked wheat germ and aqueous liver extract were found to be most potent in the factor or factors which are missing, or inadequate, in yeast.

Sandza and Cerecedo[4] also reported that the 92% alcohol extract of liver contains an unknown alkali stable factor necessary for the growth of mice; their supplements included only thiamin, riboflavin, pyridoxin, and pantothenic acid, however.

The present experiments have shown that when male mice are fed a synthetic basal diet supplemented with thiamin chloride, riboflavin, pyridoxin hydrochloride, calcium pantothenate, nicotinic acid, choline hydrochloride, ascorbic acid, inositol and p-aminobenzoic acid, the growth of the animals can be definitely improved by feeding them as an additional supplement either fresh beef liver or a concentrate which we have prepared from fresh beef liver. The feeding of the additional supplements, especially the fresh liver, gave the mice in these particular experiments a more consistently clean and good looking coat of fur.

Experimental. Feeding and Handling of Animals. Twenty-six male mice from 4 litters were placed at the age of 21 days on a basal diet of the following composition: extracted casein[12] 20, sucrose 67, salts[†] 5, cod liver oil 3, corn oil 2, and butter fat (washed and filtered) 3. The corn oil should furnish more than enough vitamin E for male mice.[14] Beginning on the following day, the animals were given the following basic supplements daily for 6 days each week: thiamin chloride 10 μg, riboflavin 25 μg, pyridoxin hydrochloride 10 μg, calcium pantothenate 100 μg, nicotinic acid 100 μg, choline hydrochloride 4 mg, ascorbic acid 800 μg,[15] inositol 3 mg, and p-aminobenzoic acid 1.5 mg. These supplements were given in 2 cc of a 5% ethyl alcohol solution placed in a special supplement cup; the regular drinking water bottles were withheld until the supplement solutions were quantitatively consumed.

---

[3] Norris, E. R., and Hauschildt, J., *Science,* 1940, **92**, 316.

[4] Sandza, J. G., and Cerecedo, L. R., *J. Nutrition,* 1941, **21**, 609.

[5] Martin, G. J., *Science,* 1941, **93**, 422.

[6] Woolley, D. W., *J. Biol. Chem.,* 1940, **136**, 113.

[7] Woolley, D. W., *Science,* 1940, **92**, 384.

[8] Woolley, D. W., *J. Biol. Chem.,* 1941, **139**, 29.

[9] Woolley, D. W., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 565.

[10] Martin, G. J., and Ansbacher, S., *J. Biol. Chem.,* 1941, **138**, 441.

[11] Carruthers, C., *Science,* 1941, **93**, 44.

In preliminary experiments, 24 mice were used to determine whether these basal rations used contained enough casein, salts and pure vitamin supplements. Six of the preliminary mice received the same diet as the control group (Group I) of the main experiment, detailed below, and showed an appearance and rate of growth similar to that which will be described for Group I; the condition and growth of the remaining 18 preliminary animals, divided into 3 groups of 6, were no better, even though one group received 30% casein and 7% salts, another double quantities of basal supplements, and the third group both the double supplements and the increased casein and salts.

By the end of 3 weeks on the basal diet and supplements, 3 of the 26 mice had died. The first died at the age of 36 days, showing paralysis of the hind legs, diarrhea, and brownish discoloration around the genitals and anus; a second died at the age of 37 days with a diarrhea; the third died at the age of 42 days, exhibiting paralysis of the hind legs, diarrhea, and brown discoloration around the anus and urethra and extending extensively up underneath the body. All 3 of these animals lost weight markedly before death.

At the age of 37 days, the 23 mice which were steadily gaining weight were divided into 4 groups which were as homogeneous as possible with regard to litters, weights of the animals at the age of 37 days, and weight gains between the ages of 21 and 37 days.

[†] Hawk and Oser salt mixture,[13] with 1.68 parts of $ZnCl_2$ and 2.00 parts of $CuSO_4 \cdot 5\ H_2O$ substituted for every 3.68 parts of NaF.

[12] Halliday, N., and Evans, H. M., *J. Nutrition,* 1937, **13**, 657.

[13] Hawk, P. P., and Oser, B. L., *Science,* 1931, **74**, 369.

[14] Bryan, W. L., and Mason, K. E., *Am. J. Physiol.,* 1940, **131**, 263.

[15] Kleiner, I. S., and Tauber, H., *Food Research,* 1936, **1**, 399.

Additional supplements were then fed daily on 6 days a week as follows:

Group I, the control group, was given no additional supplement; Group II received 2 mg of pimelic acid, increased to 10 mg when the mice were 56 days old; Group III received about a quarter gram of fresh beef liver, gradually increased until approximately a half gram was being given when the mice were 59 days old; Group IV was given a dried concentrate from an extract of fresh beef liver, to be described below. For about the first 6 weeks this liver concentrate was mixed with the daily solution of basic supplements; about 60 mg was given at first, which was gradually increased to approximately 140 mg when the mice were 57 days old. This method of giving the concentrate was not entirely satisfactory, however, since some animals showed a tendency to leave a dried residue in the supplement cups, and it was then necessary to resuspend this residue in water so that it would be entirely consumed. Accordingly, when the animals were 66 days old, the liver concentrate was finally given as 5%[‡] of the basal diet.

All the mice were killed at the age of 116 days.

Preparation of Beef Liver Concentrate. For the preparation of the liver concentrate given to Group IV, 3500 g of fresh beef liver was first freed from all tough tissue and finely ground. With this, in succession, were mixed thoroughly an equal volume of distilled water, about 40 cc of hydrochloric acid, and about 200 cc of toluene. The final pH was 4.5. After this preparation had autolyzed at 37°C for 3 weeks, 5 pounds of butyl alcohol was thoroughly mixed with it, and the entire batch warmed to about 60-70°C on a steam bath. It was then allowed to stand at room temperature over night. In the morning, the process of filtration and concentration of the filtrate *in vacuo* was begun. The residue was thoroughly extracted in a soxlet extractor, first with a mixture of butyl alcohol and distilled water and then with acetone. The acetone was removed from the latter extract by fanning in an open dish, and the combined extracts were then added to the original filtrate.

The filtrate, after having been freed from butyl alcohol and toluene by concentration to a mushy syrup *in vacuo,* was transferred to a large separatory funnel and washed with petroleum ether until the petroleum ether washing was only a light yellow. The remaining syrupy water solution with suspended solids was placed in a large evaporating dish with a little toluene and evaporated under an electric fan to a thick mush; it was then quickly and thoroughly dried in a vacuum desiccator over potassium hydroxide. The dried material was washed with petroleum ether to remove the last traces of toluene and ground to a fine powder, which was somewhat hygroscopic.

[‡] Five g of concentrate were mixed with every 95 g of the basal diet.

Effects on Growth. The growth curves of the 4 groups of mice are shown in Fig. 1. For the first 15 days of the experiments, the average growth rates and the weights did not differ appreciably. However, after the additional supplements were given Groups II, III and IV, as described above, the average growth rates of the mice in Groups III and IV were increased as compared with the growth rates of the animals in I and II, which did not differ from each other to any appreciable extent. The animals fed fresh beef liver grew better than any of the other groups; however, it is, of course, impossible to say whether there was any qualitative difference between the growth-promoting factor or factors of the fresh liver

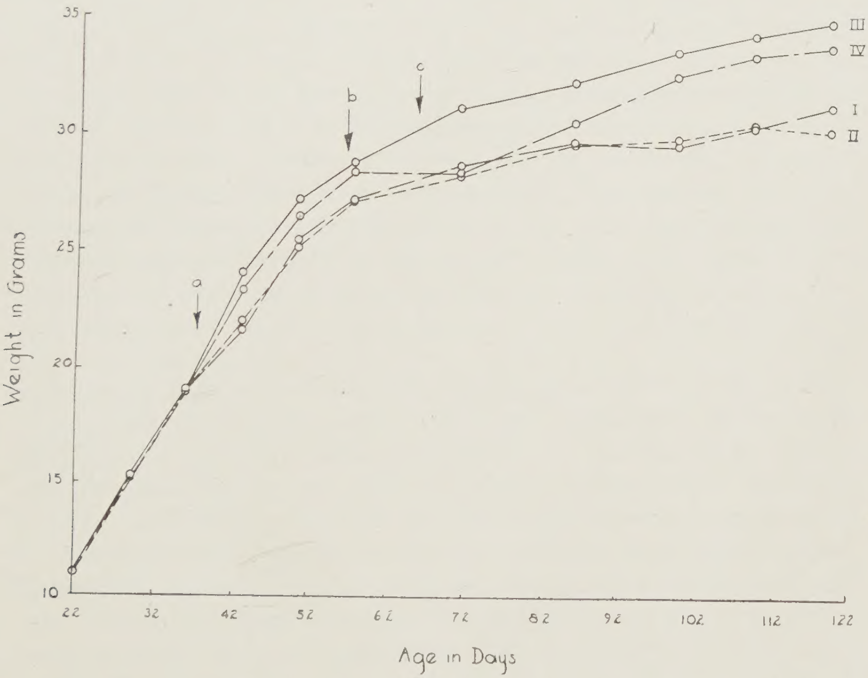


FIG. 1.

Growth curves of mice. I, 6 mice, control group; II, 6 mice, additional supplement 2 mg pimelic acid per day, later increased to 10 mg; III, 5 mice, about 0.25 g fresh liver per day, gradually increased to about 0.5 g; IV, 6 mice, about 60 mg of the liver concentrate per day, gradually increased to 140 mg and finally given as 5% of the basal diet.

a, began to give additional supplements; b, pimelic acid increased to 10 mg per day; c, liver concentrate given as 5% of basal diet.

given Group III and the factor or factors of the liver concentrate which was given to Group IV. The temporary failure of Group IV to gain weight, which was evidenced from the weights of the animals at the age of 71 days, was very probably due to the unsatisfactory method of giving the liver concentrate. Soon after the concentrate was incorporated into the basal diet, Group IV gained weight at an even more rapid rate than Group III. This very rapid weight gain shown by Group IV soon levelled off, however, and substantially paralleled the rate of weight gain shown by Group III. The final difference between the average weights of Groups III and IV may not be significant.

Appearance of Animals. All 5 mice in Group III, receiving fresh liver always possessed good looking fur and a clean appearance, with the exception of a pale yellow staining which appeared on the fur around the genitals of 3 of the animals towards the end of the experimental period. The condition of the pelage of the animals in Group IV, given the liver concentrate, was less consistently satisfactory as to cleanliness and neatness; this may have been at least partly due to the unsatisfactory method initially used for the administration of the liver concentrate. Groups I and II were the least satisfactory in average cleanliness and neatness of the pelage; pimelic acid had definitely no beneficial effect on the fur of the animals in these experiments. In Group I some alopecia developed around the genitals of one mouse; in Group II one animal showed an extensive alopecia on the face and chest, around the legs and in a large area surrounding the genitals, while the fur on the back of a second animal was very thin.

Discussion. The synthetic diet and supplements used for mice in these experiments, which covered the first 4 months of life, were inadequate to promote the most rapid rate of growth. Both fresh beef liver and a liver concentrate increased the growth rate, and the fresh liver especially improved the condition of the fur. While pimelic acid had no significant effect on growth, and certainly no beneficial effect on the fur of the animals, its value as a nutritional factor still remains undetermined, since the addition of a single nutritional factor to a synthetic ration may have no effect without the addition of other factors, or may even have an adverse effect.[16]

Summary. When fed a basal diet containing extracted casein, sucrose, salts, cod liver oil, corn oil, and butter fat, together with supplements consisting of thiamin chloride, riboflavin, pyridoxin hydrochloride, nicotinic acid, calcium pantothenate, choline hydrochloride, ascorbic acid, inositol and p-aminobenzoic acid, young mice grew at a good rate but after some weeks did not present consistently clean and good looking fur. Increasing the casein and salts or doubling the daily quota of the supplements, or both, failed to improve the appearance or growth of the mice. Feeding fresh beef liver increased the growth rate and improved the pelage. A concentrate of a liver extract had similar but not as pronounced effects. Pimelic acid had no significant effect on growth and certainly no beneficial effect on the pelage, for the time range of these experiments.

---

[16] Morgan, A. F., *Science*, **1941**, **93**, **261**.

13385 P

Acetylcholine Esterase Content of Brain Tumors.

K. A. YOUNGSTROM, B. WOODHALL AND R. W. GRAVES. (Introduced by F. H. Swett.)

From the Departments of Anatomy, Surgery, and Medicine, Duke University School of Medicine, Durham, N.C.

The acetylcholine esterase content of 50 brain tumors, and of 13 brain lesions not neoplastic in character, has been determined by the pharmacological method previously described.[1] All material was obtained immediately following removal in the neurosurgical operating room. The tissue to be examined for acetylcholine esterase content was exposed to the action of no drugs. The acetylcholine esterase activity (Q.CHE.) was determined as the number of milligrams of acetylcholine iodide hydrolized by 100 mg of tissue dry weight in one hour at 37°C and pH 7.61.

The histologic or pathologic structure of the tissue resected at operation and examined for Q.CHE. content was established by multiple sections, fixed in Cajal's formalin-ammonium-bromide solution, and after the appropriate time of fixation stained with, (1) Penfield's second modification of Rio-Hortega's silver sodium carbonate stain, (2) Rio-Hortega's silver lithium carbonate stain, and (3) Cajal's gold chloride sublimate method. Other material fixed in Zenker's fixative was stained with hematoxylin and eosin and with phosphotungstic hematoxylin.

As indicated in Table I, 8 tumors of the astroblastic and astrocytic

---

[1] Youngstrom, K. A., *J. Neurophysiol.*, 1938, **1**, 357.

TABLE I.

		Q.CHE.	
Type of tumor	No. of specimens	Range	Avg
Astrocytoma (cerebellum)	4	10.7-42	
Astroblastoma (cerebellum)	4	8.1-23	
	—8		18.8
Astrocytoma (cerebrum)	3	0.8-18.2	
Gliobastoma multiforme (cerebrum)	21	1.1-24.2	5.2
Medulloblastoma (cerebellum)	7	1.1- 6	3.6
Acoustic neurinoma	2	3.6- 3.8	
Meningioma	2	2.0- 2.6	
Oligodendroglioma	2	1.0- 5.0	
Spongioblastoma polare	1	1.0	
Neurocytoma	1	0.96	
Neuroblastoma	1	3.7	
Metastatic carcinoma	1	0.8	
Metastatic melanosarcoma	1	1.9	

types occurring in the cerebellum had an average Q.CHE. of 18.8. One tumor of this type occurring in the cerebrum had a Q.CHE. of 18.2, but 2 others listed as astrocytomas had a Q.CHE. of 0.8 and 1.9 respectively. However, these last 2 were clearly of a different histologic character from the first and may very well be examples of what Scherer[2] refers to as "malignant dedifferentiation of an astroblastoma into a glioblastoma." Histological diagnosis in such cases is notably difficult and uncertain. It is also of interest to recall that a significant clinical difference is recognized between the astrocytomas of the cerebrum and those of the cerebellum. Tumors of the astroblastic and astrocytic type occurring in the cerebellum are found chiefly in children and young adults. They are well circumscribed and when completely resected may be controlled permanently from a clinical point of view. On the contrary, tumors of similar cell structure arising in the cerebrum usually occur in adults and have a poor prognosis, facts recently emphasized by Davidoff.[3] Scherer[2] has pointed out in addition the striking morphologic differences between the diffuse growth of the cerebral astrocytomas and the localized development of the cerebellar tumors of similar cell type.

In 21 tumors of the cerebrum conforming to the structure of glioblastoma multiforme the Q.CHE. ranged between 1.1 and 24.2, Av. 5.2. Only 4 had a Q.CHE. greater than 6. The histologic variation in this type of malignant tumor of the adult cerebrum is well recognized and of special interest in view of the wide variation of Q.CHE. found in the specimens examined.

Seven medulloblastomas of the cerebellum, a tumor of uniform cell type and of recognized malignancy, showed a Q.CHE. between 1.1 and 6.0 with an average of 3.6. This contrasts markedly with the astroblastic and astrocytic tumors of the cerebellum.

---

[2] Scherer, H. J., *Brain*, 1940, **63**, 1.

[3] Davidoff, L. M., *Arch. Neurol. and Psychiat.*, 1940, **44**, 1246.

Eleven varied tumors of the brain embracing the last eight types listed in the table had a Q.CHE. between 0.8 and 5.0 with an average of 2.4.

From these studies it seems apparent that the astrocyte and its progenitor the astroblast are the cell types most intimately associated with cholinesterase formation in neoplastic brain tissue. In favor of this view is the high cholinesterase content of the astrocytomas and astroblastomas and the distinctly low values obtained for practically all other types of brain tumors. Notable exceptions to this would appear to be an occasional glioblastoma multiforme with a high cholinesterase content and the 2 astrocytomas of the cerebrum which had a low Q.CHE. No satisfactory explanation of these discrepancies is available for this preliminary report on the problem.

It is also of considerable interest to note that tumors such as neurocytomas and neuroblastomas have shown a very low Q.CHE. Although some nerve cells are known to liberate acetylcholine, they do not necessarily also form cholinesterase. The unique experiments of Boell and Nachmansohn[4] lend further support to this view.

The intimate relationship between the astrocyte and cholinesterase is also suggested by their simultaneous appearance during the development of the human cerebrum.[5, 6] Data are not available for comparison of other regions in this regard. The work is being continued and will be reported more fully in another place.

---

[4] Boell, E. J., and Nachmansohn, D., *Science,* 1940, **92**, 513.

[5] Kershman, J., *Arch. Neurol. and Psychiat.*, 1938, **40**, 937.

[6] Youngstrom, K. A., *J. Neurophysiol.*, 1941, **4**, 473.

13386 P

Reactions of Monkeys to Experimental Respiratory Infections.* I. Response to Influenza Virus A.

O. C. WOOLPERT, J. L. SCHWAB, S. SASLAW, C. MERINO AND C. A. DOAN.

From the Departments of Bacteriology and Medicine, Ohio State University, Columbus.

Most of our current knowledge of experimental influenza has been derived from observations made on small laboratory animals, particularly mice and ferrets. Vieuchange[1] reported certain manifestations of infection in 2 monkeys inoculated with an unfiltered mouse-lung suspension of the "W.S." strain of virus. Some 2 years ago we began a series of correlated studies—clinical, hematological, bacteriological, and immunological—in normal monkeys inoculated intranasally: (1) with the virus of influenza A, (2) with *Streptococcus hemolyticus* Group C, and (3) with the virus and streptococcus in various sequences.[2] Similar studies are in progress, utilizing vitamin-deficient monkeys. This communication records the reaction of normal monkeys to the virus alone.

Methods. The PR-8 strain of influenza virus used was maintained by intranasal passage in young adult white mice. The inoculum for the monkeys was prepared as follows: The lungs of infected mice were triturated in 9 volumes of buffered saline; the suspension was filtered through Berkefeld V candles and the filtrate stored at −78°C; after a period of stabilization it was titrated in mice and was retested for maintained virulence from time to time during experimentation.

Young adult monkeys *(Macaca mulatta)* were held under quarantine during a period of preliminary laboratory tests. After suitable base-line observations were completed the animals were anesthetized with ether and 3 cc of the prepared material (10,000 mouse mld) were dropped into the external nares.

Complete serial blood-cell studies, repeated tests for the presence of virus in nasal and throat washings, and serum-neutralization tests were continued over a period of several weeks.

---

* Recognition is herewith accorded to a grant from the Comly Research Fund in the furtherance of these studies.

[1] Vieuchange, M. J., *Acad. de med.*, Paris, 1939, **121**, 100.

[2] Doan, C. A., Woolpert, O. C., Merino, C., Schwab, J. L., and Saslaw, S., *Proc. Am. Soc. Clin. Invest., J. Clin. Invest.*, 1941, **20**, 452.

Results. None of the 7 monkeys thus inoculated intranasally with the influenza virus manifested clinical symptoms of infection. That invasion regularly occurred, however, was proved: (1) by consistent changes in the blood-cell equilibria, (2) by the development of neutralizing antibodies.

The blood-cell response was uniformly prompt, definite and fairly consistent. The total white-cell count characteristically fell within 24-48 hours; for example, in monkey No. 2 (Fig. 1) from 20,000 to 12,000 with granulocytes reduced from 13,400 to 6,700; the lowest granulocyte-level, 1,920, was recorded on the 6th post-inoculation day, at which time the lymphocytes had shown a reciprocal rise from 6,000 to 9,800. In another animal, No. 3, the total leucocytes were reduced from the preinoculation level of 25,000 to 17,000 and the neutrophilic granulocytes from 17,500 to 8,500 during the first 24 hours; the lowest granulocyte-level, 3,700, was recorded on the 5th post-inoculation day. The monocytes showed no significant fluctuations. The red blood cells and hemoglobin remained essentially unaltered.

In general it was observed that each time the granulocytes decreased there was a reciprocal rise in the lymphocytes, particularly striking between the 1st and 6th post-inoculation days, a phenomenon

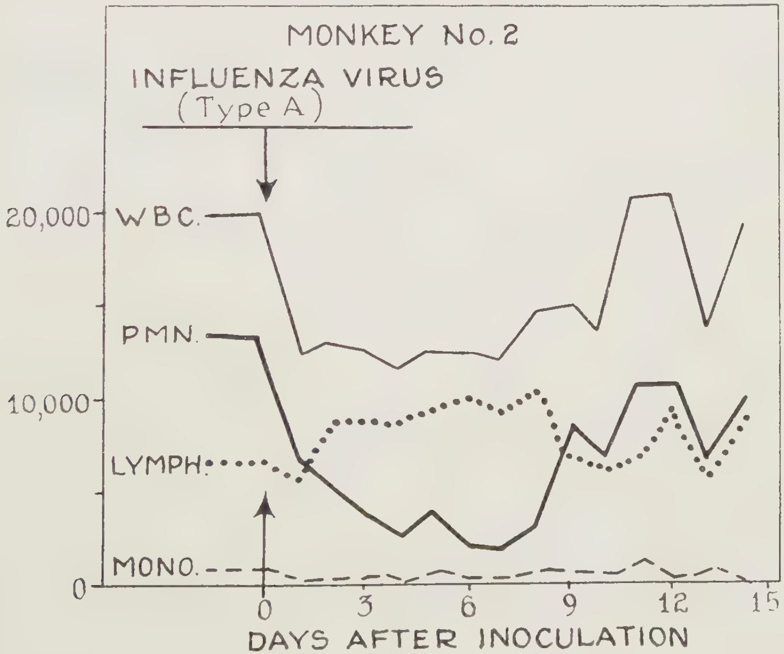


FIG. 1.

not infrequently observed in other studies of hemocytological disequilibria reported and commented upon previously.[3]

Virus-neutralizing antibodies, as evidenced by mouse-protection tests, were demonstrable in the serum of the six monkeys so tested, as early as the 8th day—approximately coincident with the beginning of spontaneous recovery from the granulocytopenia—and in every case by the 14th post-inoculation day. Repeated daily attempts to recover the virus from nasal and throat washings as well as from the blood were non-productive.

It may be concluded on the basis of the altered blood picture and from the specific antibody-response, that the monkey reacts characteristically to influenza A virus following intranasal inoculation. However, individuals of this species appear to be more resistant clinically than the smaller experimental animals ordinarily employed in studies of influenza, and while showing certain individual variations, they are probably more resistant to this disease, when uncomplicated, than the natural host, man. The monkey presents certain advantages over the small animals since its reactions, particularly the hematological and serological, may be more accurately followed.

13387 P

Reactions of Monkeys to Experimental Respiratory Infections.* II. Response to *Streptococcus hemolyticus* Group C.

J. L. Schwab, S. Saslaw, O. C. Woolpert, C. Merino and
C. A. Doan.

From the Departments of Bacteriology and Medicine, Ohio State University, Columbus.

Comparatively few observations have been made of experimentally induced streptococcal infections of the respiratory tract in monkeys. Blake and Cecil[1] cite a sharp leucocytic rise within 24 hours following an intratracheal inoculation of hemolytic streptococcus, and in the lungs observed vascular engorgement, interstitial edema, and leucocytic infiltration. This report deals with the clinical, hematological, bacteriological, and immunological findings in monkeys inoc-

---

[3] Wiseman, B. K., Doan, C. A., and Erf, L. A., *J. A. M. A.*, 1936, **106**, 609.

* Recognition is herewith accorded to a grant from the Comly Research Fund in the furtherance of these studies.

[1] Blake, G., and Cecil, L., *J. Exp. Med.*, 1920, **32**, 401.

ulated intranasally with *Streptococcus hemolyticus,* Lancefield Group C.

Methods. Healthy young adult monkeys *(Macaca mulatta),* selected for study, were isolated and observed for a period of 2-3 weeks during which time base lines for the various determinations were established. Animals found to be in satisfactory equilibrium were then inoculated intranasally while under deep ether-anesthesia with 3 cc of a saline suspension of living organisms (250 millions per cc). The animals were then observed daily for a period of from 3 weeks to 9 months.

Results. Clinical Characteristics. A majority of the monkeys thus studied developed distinct clinical signs of infection, including fever, anorexia, and loss of weight. Although most of the animals recovered promptly, several developed chronic foci with persistently positive throat-cultures for the Group C streptococcus.

Hematology. The first sign of invasion always appeared within 24 hours of inoculation as an acute, specific, polymorphonuclear leucocytosis. For example, in monkey No. 6 (Fig. 1), the white count rose from 17,000 to 51,000 overnight. The neutrophils usually maintained this initial high response for not longer than 24 hours, characteristically falling as precipitously as they had risen. Frequently, however, several polymorphonuclear rebounds of gradually decreasing amplitude occurred during the following 10-15 days (Fig. 1) before the previous cellular equilibrium was reëstablished, thus reflecting a successful cellular defense against the bacterial invasion. The lymphocytes showed minor reciprocal fluctuations,[2] corresponding to the polymorphonuclear peaks. The red cells and hemoglobin fell progressively with rising reticulocytes until the infection was brought under control, when spontaneous recovery followed.

Opsonocytophagic Index. Daily determinations based on a modification of Huddleson's method, revealed no significant increase in the opsonocytophagic index for Group C streptococci during the entire acute episode.

Antistreptolysin-titers. In only one monkey did the antistreptolytic level rise (from 10 units on the 5th to 200 units on the 15th day of infection), the remaining animals showing no significant variations.

Precipitin-titers. As a rule, in the monkeys exhibiting low antistreptolysin titers, little or no demonstrable precipitin was evident. In this group when a positive precipitin reaction occurred it ap-

---

[2] Woolpert, O. C., Schwab, J. L., Saslaw, S., Merino, C., and Doan, C. A., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 558.

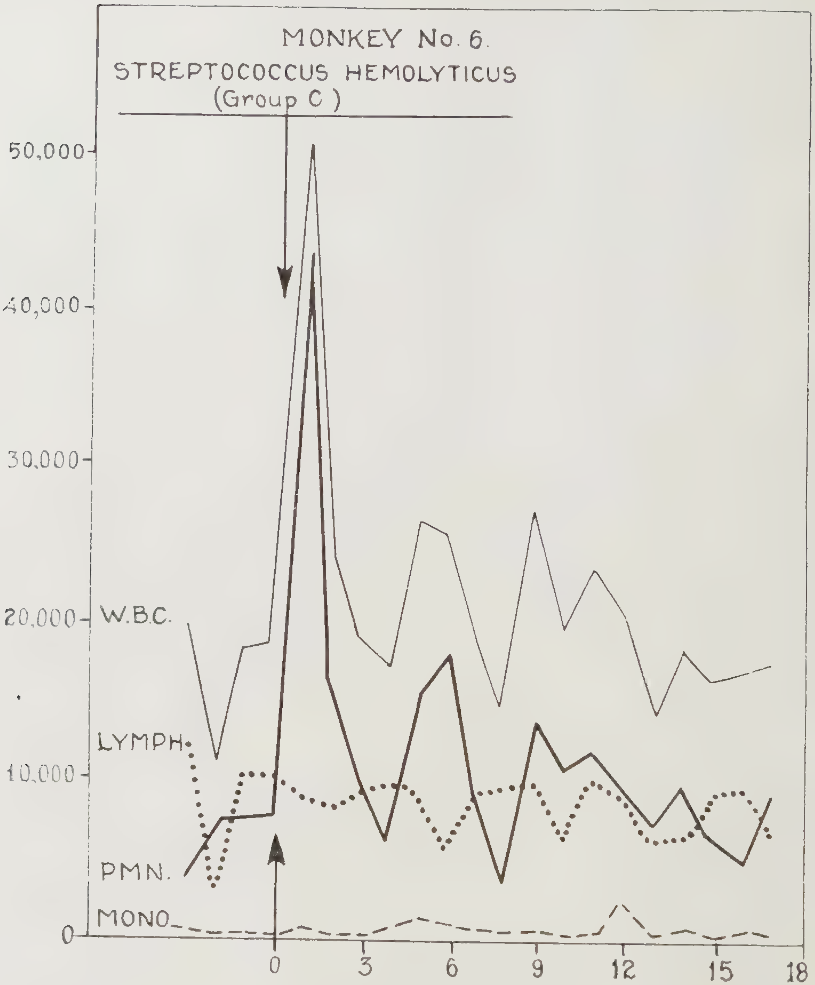


FIG. 1.

peared only with undiluted antigen, and not before 5 days after inoculation, when weak reactions were occasionally observed.

Bacteriological. Throat cultures were taken daily and revealed hemolytic streptococci on most of the examinations during the period of acute invasion. The streptococci isolated were typed by Lancefield's method to insure identification and to exclude possible spontaneous contamination by other strains from human contacts. Blood cultures were negative for streptococci in all instances.

Summary. The response of monkeys to primary intranasal inoculation with *Streptococcus hemolyticus,* Group C, was characterized chiefly by a prompt but fleeting polymorphonuclear leucocytosis with occasional minor recurrent granulocytic elevations, followed by a transitory anemia. The opsonocytophagic index, antistreptolysin and precipitin titers were not significantly affected. Clinical manifestations of disease were mild but definite, and it is concluded that the cellular reaction, aided, if at all, only in slight degree by humoral factors, was the principal demonstrable defensive mechanism which these monkeys mobilized in successfully opposing the initial invasion by streptococci via the respiratory tract.

13388 P

Reactions of Monkeys to Experimental Respiratory Infections. III. Response to Mixtures of Influenza Virus and Streptococcus.*

C. Merino, C. A. Doan, O. C. Woolpert, J. L. Schwab and S. Saslaw.

From the Departments of Medicine and Bacteriology, Ohio State University, Columbus.

In earlier studies[1] employing mice, a shorter survival period and a greater proportion of deaths were observed in those animals which received mixtures of *Streptococcus hemolyticus,* Group C, and influenza-A virus, than in those receiving only one agent alone. The present report records particularly the hematological reactions in monkeys in which one infection was superimposed upon the other via the intranasal route.

Methods. The preparation of inocula, the dosages employed, and the method of administration were carried out as previously described.[2, 3] When the two agents were given simultaneously the concentrations were so adjusted that 3 cc of the mixture contained the full dosage of each agent.

Results. Clinical Characteristics. All monkeys receiving both agents, in whatever order, survived the infections and manifested only minimal clinical evidences of disease, except monkey No. 3

---

* Recognition is herewith accorded to a grant from the Comly Research Fund in the furtherance of these studies.

[1] Schwab, J. L., Blubaugh, F. C., and Woolpert, O. C., *J. Bact.*, 1941, **41**, 59.

[2] Woolpert, O. C., Schwab, J. L., Saslaw, S., Merino, C., and Doan, C. A., Proc. Soc. Exp. Biol. and Med., 1941, **48**, 558.

[3] Schwab, J. L., Saslaw, S., Woolpert, O. C., Merino, C., and Doan, C. A., Proc. Soc. Exp. Biol. and Med., 1941, **48**, 560.

(Fig. 1-D), which became acutely ill and died with an overwhelming streptococcal septicemia.

Experiment 1. *Simultaneous Inoculation with Virus and Streptococci.* The 2 monkeys receiving a mixture of virus and streptococci, each manifested within 24 hours a marked polymorphonuclear leucocytosis. The total white blood cells increased from 12,000 to 56,000 (granulocytes 4900 to 44,300) in No. 7 (Fig. 1-A), and from 13,000 to 39,000 (granulocytes 8800 to 33,000) in No. 8. Both animals, between the 11th and 15th days after inoculation, developed a definite granulocytopenic leucopenia with, in one instance, a relative, and in the other an absolute lymphocytosis, suggesting a masked and *delayed* reaction to virus. Specific neutralizing antibodies were demonstrated.

Experiment 2. *Inoculation with Streptococcus Followed by Virus.* Four monkeys (Nos. 9, 10, 12, 13) receiving a primary inoculation of hemolytic streptococci, developed the usual leucocytosis within 24

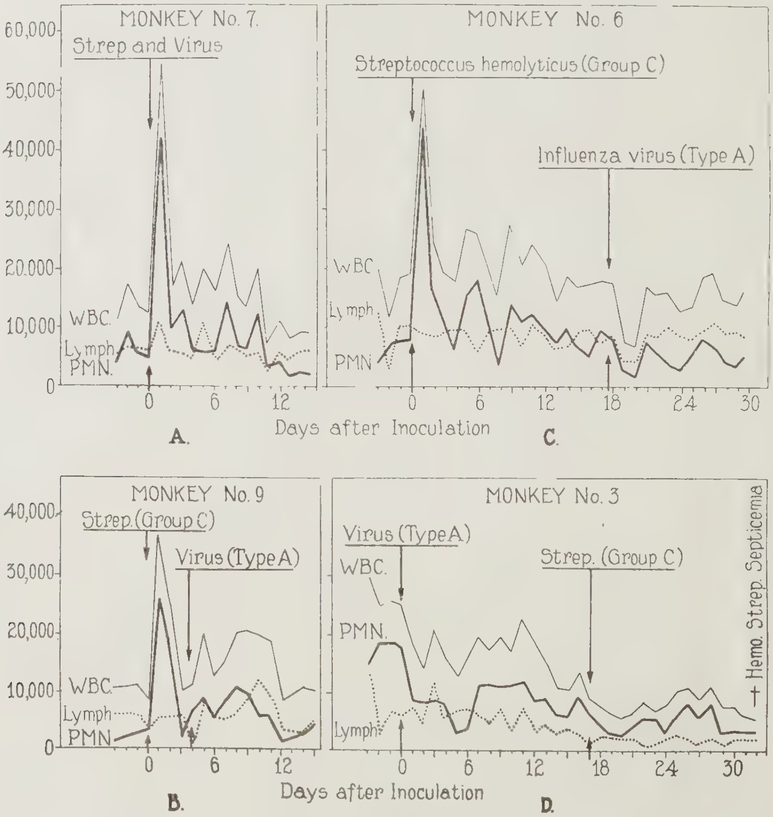


FIG. 1.

to 48 hours (Fig. 1-B) but, when inoculated 4 days later with the virus, *only one of the animals showed the typical inhibition of granulocytes which has characterized all initial, uncomplicated viral infections.*

Three monkeys (Nos. 1, 5, 6), which also responded characteristically with a marked neutrophilic leucocytosis to the primary inoculation with streptococci, when inoculated secondarily with influenza virus 15-17 days later, all developed a definite relapsing leucopenia within 1 to 3 days (Fig. 1-C), quite independently of, and apparently uninfluenced by, the original streptococcal infection.

Experiment 3. *Inoculation with Virus Followed by Streptococcus.* Four monkeys (Nos. 15, 16, 17, 18) were infected with influenza virus and 4 days later streptococci were superimposed intranasally. Two of the monkeys (Nos. 15 and 17) responded to the primary viral inoculation with a profound granulocytopenia (7800 to 1950, and 15,600 to 3230, respectively); the former, thereafter, showed an almost identical depression in neutrophils during the 48-hour period following hemolytic streptococcal inoculation (8400 to 1920), while the latter revealed a striking neutrophilic leucocytosis (8800 to 33,000), typical of primary pyogenic stimulation; no significant leucopenia developed in the other 2 monkeys prior to the superimposed streptococcal inoculation, and in one instance a moderate leucocytosis occurred (No. 16—3300 to 17,500) while in the other, both cellular and clinical reactions were negligible to virus and streptococci alike.

Three monkeys (Nos. 2, 3, 4) were inoculated with streptococci 15 to 17 days following the primary infection with influenza virus. A definite leucopenia developed in all within 3 to 4 days after the viral inoculation, as illustrated in Fig. 1-D, but *in none of the 3 did a leucocytosis, typical of initial streptococcal infection follow.* Monkey No. 3 became progressively sicker and died with hemolytic streptococcal sepsis on the 32d day following exposure to virus and the 15th day after the streptococcal complication had been introduced. Type C streptococci were recovered from all organs at postmortem.

13389 P

Reactions of Monkeys to Experimental Respiratory Infections. IV. Response to Re-inoculation with *Streptococcus hemolyticus.**

C. A. Doan, S. Saslaw, M. Beard, O. C. Woolpert, J. L. Schwab
and C. Merino.

From the Departments of Medicine and Bacteriology, Ohio State University, Columbus.

The various reactions of *Macaca mulatta* to primary intranasal inoculation with *Streptococcus hemolyticus,* Group C, alone or in conjunction with the virus of influenza A, have been described in preceding communications.[1, 2, 3] A study of the same phenomena on reinfection with the same strain of hemolytic streptococci is the basis of this report.

Methods. Eight monkeys were inoculated intranasally with streptococci 2 to 3 months previously. Six had survived also exposure to influenza A virus incurred either shortly preceding or just following the streptococcal episode. Before reinoculation, the recovered monkeys were observed and studied intensively over a control period of at least 1 to 2 weeks. *Streptococcus hemolyticus,* Group C, was then administered intranasally under ether-anesthesia in the same dosage (750,000,000 organisms in 3 cc saline) as was used for the primary inoculations.

Three monkeys, not included in the above group but having apparently recovered from a primary inoculation with hemolytic streptococci, redeveloped positive throat cultures for Lancefield Group C streptococci some 46 to 56 days after inoculation. Coincident with the spontaneous exacerbation of a latent autogenous focus *or* due to reinfection from others in our monkey colony, certain symptoms and signs appeared.

Results. Only 2 of the 8 reinoculated animals in the first group presented recognizable clinical evidences of disease. Symptoms and signs suggestive of renal damage appeared 2 weeks after reinoculation (95 days after primary inoculation) in one animal, and 4 days after reinoculation (108 days after primary inoculation) in another.

---

* Recognition is herewith accorded to a grant from the Comly Research Fund in the furtherance of these studies.

[1] Woolpert, O. C., Schwab, J. L., Saslaw, S., Merino, C., and Doan, C. A., Proc. Soc. Exp. Biol. and Med., 1941, **48**, 558.

[2] Schwab, J. L., Saslaw, S., Woolpert, O. C., Merino, C., and Doan, C. A., Proc. Soc. Exp. Biol. and Med., 1941, **48**, 560.

[3] Merino, C., Doan, C. A., Woolpert, O. C., Schwab, J. L., and Saslaw, S., Proc. Soc. Exp. Biol. and Med., 1941, **48**, 563.

General malaise, anorexia, hypertension, edema of the face, legs, and perineal region, and albuminuria, with pus cells, red cells and casts in the urinary sediment were found. Identical phenomena were noted in all 3 of the monkeys showing the spontaneous reappearance of Group C streptococci in their respective throat cultures. Experimental reinoculation of these 3 monkeys with Group C streptococci 2 months after subsidence of the acute spontaneous renal manifestations, failed to elicit any further constitutional signs.

Throat cultures from those monkeys which became acutely ill following reëxposure to *Streptococcus hemolyticus*, remained consistently positive for this organism, while streptococci were found only irregularly and transiently in the throats of other animals. Blood cultures were negative in all instances. The antistreptolysin titer rose significantly in 4 of 8 animals tested. The precipitin level also showed a sharp rise and the titer was best maintained in those

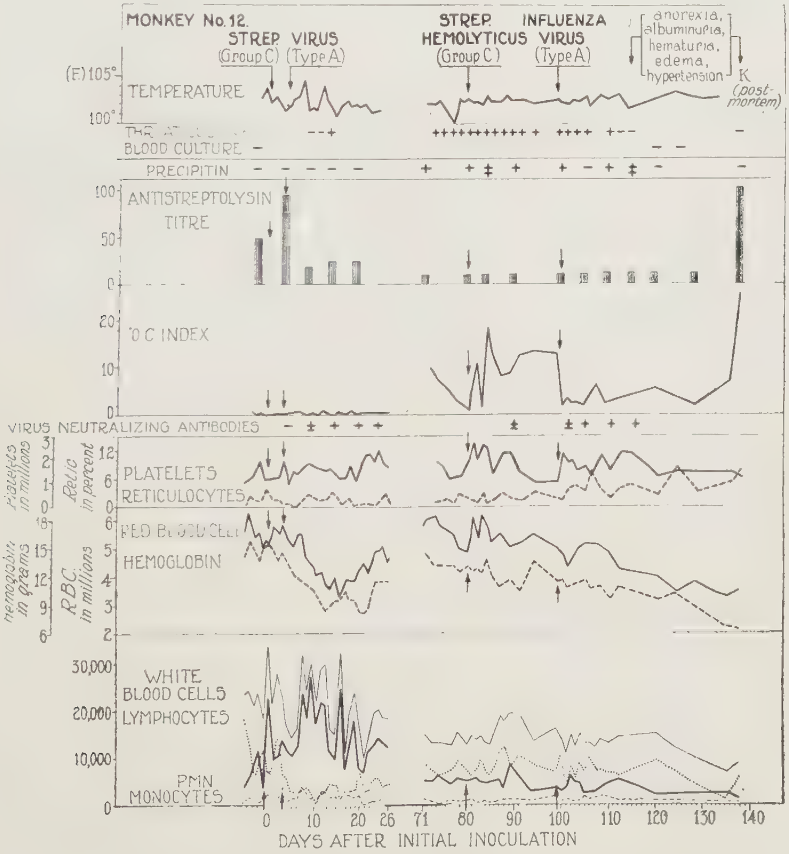


FIG. 1.

monkeys which handled the reinfections most effectively. Most noteworthy was the marked increase in opsonocytophagic index evoked by reinoculation with streptococci. On the other hand, *the granulocytosis which has proved to be one of the most characteristic responses to primary streptococcus infection*[2] *was wholly lacking in all reinoculated animals.*

Fig. 1 summarizes and correlates the pertinent data in one representative individual in this reinoculated series. In monkey No. 12, neutrophilcytosis with reciprocal lymphocytopenia, continued throughout the first 20 days of acute reaction to the original streptococcal inoculation, the superimposed influenza virus having little if any granulocyte-inhibiting influence. The dominance of the streptococcal element in this syndrome is further indicated by the progressive hemolytic anemia that developed during the first 12 to 20 days, beginning erythrocyte-recovery coinciding with subsidence of the granulocytosis. Specific precipitins and the opsonocytophagic index for Group C streptococci were essentially negative. By the 71st day all cellular equilibria had been reëstablished and monkey No. 12 was in good clinical condition. Intranasal reinoculation with hemolytic streptococci was done on the 80th day. In sharp contrast to the earlier phenomena, no granulocytosis occurred, *but* a prompt and significant compensatory increase in the opsonocytophagic index was noted. This high opsonic index persisted until it was suddenly inhibited or neutralized when the influenza virus was once again instilled into the nares. Granulocytes, lymphocytes, red cells, and hemoglobin fell steadily thereafter. By the 108th day there appeared signs of renal damage with hypertension (systolic 185 mm, diastolic 140 mm Hg) albuminuria (100 mg %), hematuria, casts and edema.

13390 P

Lack of Immunity to Latent Virus.

Gustave Freeman. (Introduced by G. F. Dick.)
From the Department of Medicine, University of Chicago, Chicago, Ill.

The apparent failure to encounter immune mice in the spontaneous mouse infections described by Dochez, *et al.*,[1] and us[2,3] suggested this attempt to produce evidence of immunity experimentally. It is of particular interest because the host-virus relationship seemed to be latent until provoked experimentally. The virus then grew highly virulent, killing all mice that became sick. None of the surviving mice appeared to be immune to the latent virus.

---

[1] Dochez, A. R., Mills, K. C., and Mulliken, B., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 683.

[2] Gordon, F. B., Freeman, G., and Clampit, J. M., Proc. Soc. Exp. Biol. and Med., 1938, **39**, 450.

[3] Freeman, Gustave, *Arch. Path.*, 1940, **29**, 505.

Approximately 400 mice obtained at different times from a Missouri breeder were observed for varying periods of time up to 14 months. These stock mice appeared to be well although they were known to carry the virus. None of these mice died spontaneously with pneumonitis.

Mice were anesthetized with ether and inoculated intranasally with 1:20 suspension of infected lung emulsion in saline. Lungs of sick mice were cultured on blood agar plates and 30% ascitic fluid agar, aerobically and anaerobically. Occasional colonies appeared from lungs of moribund animals but no organisms grew consistently enough to have etiological significance. Pleuropneumonia-like organisms[4, 5] were sought particularly since these have confused work with viruses in mice.

Groups of mice were inoculated by various routes in an attempt to immunize them. The survivors were reinoculated intranasally with virus 30 days after the initial attempts in order to test for immunity. The results are seen in Table I.

During one period of observation about 50% of the stock mice became ill and died with the disease after intranasal inoculation of sterile physiological saline. Animals that did not die after such treatment were found, nevertheless, to be susceptible to the disease by inoculation with virus. At other times saline seldom affected the mice. They were all susceptible to the disease, however, when inoculated with virus.

The disease is propagated by the passage of lungs of stock mice intranasally in series, the disease appearing usually within the first 3 passages. Groups of 3 were passed in this manner. One animal of each group was used to inoculate the succeeding group of three until

TABLE I.

Route	No. of mice	Surviving 30 days	Surviving reinoculation intranasally
Intracranial	6	6	0
Subcutaneous	6	6	0
Intraperitoneal	6	6	0
Intranasal	6	0	—
” (saline)	6	2	0

---

[4] Klieneberger, E., *J. Hyg.*, 1938, **38**, 458.

[5] Sabin, A. B., *Science*, 1939, **89**, 228.

TABLE II.

Dilution	Days alive									Survivors reinoculated (43rd day)	Days alive after reinoculation				Survivors
	2	3	5	7	8	10	13	14	15		2	3	4	14	
1-20	4	2								0					—
10^{-2}	2	2		1					1	0					—
10^{-3}			2	2	1					1		1			0
10^{-4}				2	1	2	1			0					—
10^{-5}								1	1	4		2	1	1	0
Saline										6	4	1	1		0

the disease occurred uniformly in all inoculated animals. The survivors were inoculated subsequently with known virus and succumbed to the disease. None proved to be immune.

Mice were inoculated with graduated dilutions of virus. Survivors of the sublethal doses were available for testing. These animals were reinoculated with lung emulsion of the lowest dilution 43 days after the original inoculations. The results are recorded in Table II.

Uninoculated cagemates of sick and moribund mice did not contract the disease. These animals, nevertheless, demonstrated no immunity to virus inoculation.

Large quantities of virus were injected subcutaneously and intraperitoneally into rabbits weekly for 8 weeks. Antiserum was mixed with virus for 30 minutes and inoculated intranasally one week after the last injection. The activity of the virus was not neutralized. Neither was previous intraperitoneal injection of antiserum effective in protecting mice against the virus.

This report describes the unusual immunological phenomenon of an invariably fatal clinical infection caused by a virus that lies dormant in the same host until stimulated by serial passage of lung emulsion or of physiological saline inoculated intranasally. No evidence of immunity to the latent virus has been demonstrated.

13391

Effect of Iodoacetate and Malonate on Respiration of Sea Urchin Sperm.

E. S. Guzman Barron and J. M. Goldinger.

From the Lasker Foundation for Medical Research, The University of Chicago, and Woods Hole Marine Biological Laboratory.

Since Lundsgaard[1] discovered that iodoacetate in small concentrations inhibited the fermentation of glucose by yeast without affecting its respiration, numerous papers have been published on the inhibitions produced by iodoacetate (of oxidation enzymes and of proteolytic enzymes), the inhibiting effect being probably due to combination with active sulfhydryl groups of the protein component of the enzymes. In some instances, concentrations of iodoacetate sufficient to inhibit oxidation reactions have had no effect on oxidations produced by bacteria[2] but in no case has there been reported increased respiration due to iodoacetate. The inhibition of succinate oxidation by malonate was discovered by Quastel and Whetham;[3] malonate was used as a specific inhibitor for this oxidation, though a number of investigators have reported that it inhibits also other oxidations.[4, 5, 6] Malonate seems to be utilized by moulds[7] and some bacteria;[8] in no case has it been reported that malonate is oxidized or increases the respiration of animal tissues. On studying the metabolism of sea urchin spermatozoa, we have found that both iodoacetate and malonate, used at the concentrations required for their inhibiting effect, increase their respiration.

Effect of Iodoacetate. 0.001 *M* iodoacetate dissolved in sea water, neutralized, and brought to the pH value of sea water, increased the oxygen consumption of sea urchin sperm soon after its addition (Fig. 1). This increase was due neither to oxidation of acetate (acetate produced no increase in the O_2 uptake of sea urchin sperm) nor to the action of liberated iodine (iodine inhibited the respiration). The increased O_2 uptake seems to involve the whole respiratory process, for it was accompanied by a similar increase in the CO_2 output. The R.Q. of sea urchin sperm, which was 1.08, remained approximately the same on addition of iodoacetate (1.12), the increase in the O_2 uptake in these experiments being 216% and the increase in CO_2 output 242% (Table I). Since the respiration of sea urchin sperm, like the sperm from other animals, increases on dilution, the effect of iodoacetate on sperm at three different dilutions was studied. Iodoacetate added to thick suspensions of sperm (Sperm I) increased the O_2 consumption to 277%; on approximately fourfold dilution (Sperm II), the increase was diminished to 215%; on further dilution of approximately eightfold (Sperm III) it was

---

[1] Lundsgaard, E., *Biochem. Z.*, 1930, **217**, 162.

[2] Barron, E. S. G., and Friedemann, T. E., *J. Biol. Chem.*, 1941, **137**, 593.

[3] Quastel, J. H., and Whetham, M. D., *Biochem. J.*, 1925, **19**, 525.

[4] Greville, G. A., *Biochem. J.*, 1936, **30**, 877.

[5] Weil-Malherbe, H., *Biochem. J.*, 1937, **31**, 299.

[6] Baumann, C. A., and Stare, F. J., *J. Biol. Chem.*, 1940, **133**, 183.

[7] Walker, T. K., Subramanian, V., and Challenger, F., *J. Chem. Soc.*, 1927, 3044.

[8] Aubel, E., *Compt. Rend. Acad. Sci.*, 1921, **173**, 179.

only 93% (Table II). Iodoacetate at this concentration had no effect on the respiration of sea urchin fertilized eggs; at higher concentrations (0.03 *M*) Runnström[9] reported 34% inhibition, observed only after 30 minutes following addition of iodoacetate. As a rule, there is the same delay in the inhibiting effect in other cells.[10]

Effect of Malonate. Malonate (0.03 *M*) added to sea urchin sperm increased their respiration, like iodoacetate, soon after addition, the increase being not only in the O_2 uptake but also in the CO_2

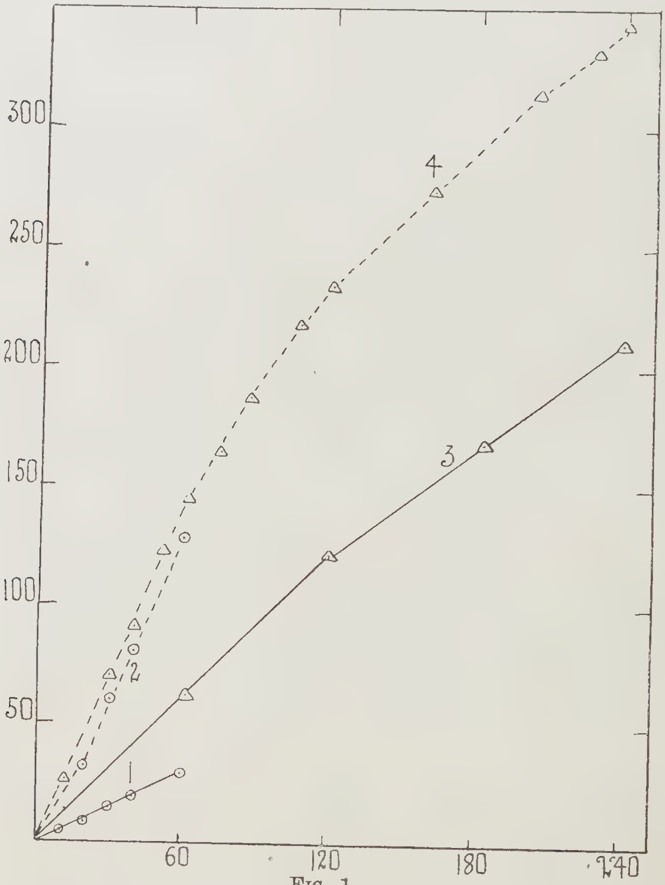


FIG. 1.

Effect of iodoacetate on the O_2 uptake of sea urchin sperm. Abscissa, time in minutes. Ordinate, O_2 uptake in mm3.

1—Control (heavy suspension).
2—Heavy suspension with iodoacetate (0.001 *M*).
3—Dilute suspension.
4—Dilute suspension with iodoacetate.

---

[9] Runnström, J., *Biol. Bull.*, 1935, **49**, 351.

[10] Barron, E. S. G., and Hastings, A. B., *J. Biol. Chem.*, 1933, **100**, 155.

TABLE I.
Effect of Iodoacetate (0.001 *M*) and of Malonate (0.03 *M*) on the Respiratory Quotient of Sea Urchin Sperm.

	O_2 uptake per hr, mm^3	CO_2 output per hr, mm^3	R.Q. $CO_2 : O_2$
Control	51.5	55.7	1.08
Iodoacetate	161.7	180.8	1.12
Control	40.0	45.0	1.12
Malonate	119.0	125.0	1.05

TABLE II.
Effect of Iodoacetate (0.001 *M*) on the Respiration of Sea Urchin Sperm and Eggs (fertilized).
Each number is the average of 5 experiments. Temp. 25°.

	O_2 uptake per hr		
Cell	Control, mm^3	$CH_2ICOONa$, mm^3	Increase, %
Sperm I	24	90.5	277
’’ II	51.5	161.7	215
’’ III	121.0	234.0	93
Eggs I	33.0	30.0	None

output. The R.Q. thus remained unchanged (Table I). As in the iodoacetate experiments, the increase in the O_2 uptake diminished on dilution of the sperm. Thus, in thick suspensions of sperm (Sperm I, Table III) malonate increased the O_2 uptake by 200%; on about threefold dilution (Sperm II) the increase was 170%; on eightfold dilution the increase was only 69%. Succinate, which is oxidized by the sperm, increased the O_2 uptake by 35%; addition of malonate plus succinate gave not an additive increase but only the increase due to malonate. Whether this was due to inhibition of succinate oxidation by malonate was not determined. In sharp contrast to the effect on sperm respiration, malonate added to fertilized eggs inhibited slightly their O_2 consumption (8%) (Table III). Oxidation of malonate by sperm seems improbable not only because its complete oxidation would result in a change of the R.Q. of sperm (R.Q. for complete oxidation of malonate, 1.50) but also because, as already indicated, acetate, the decarboxylation product of malonate, does not increase the O_2 uptake of sperm.

Discussion. Three facts bear consideration in these experiments: (1) that iodoacetate and malonate increase equally the O_2 uptake and the CO_2 output of sea urchin sperm; (2) that the effect is immediate, contrary to the delayed effect when they act as inhibitors of cell oxidations; (3) that the percentage increase diminishes on dilution of sperm. These facts have led us to assume that the respiration

TABLE III.

Effect of Malonate (0.03 *M*) on Respiration of Sea Urchin Sperm and Eggs (fertilized).

Each number is the average of 5 experiments. Temp. 25°.

Cell	O_2 uptake per hr Control, mm^3	 Malonate, mm^3	Increase, %
Sperm I	40	120	200
” II	88.4	238	170
” III	100.2	169	69
” succinate	135	171	26
Eggs I	44.5	40.7	None

of sea urchin sperm is normally inhibited by sulfhydryl groups present in the sperm fluid. The respiration would then be increased by a decrease of sulfhydryl concentration, accomplished partly by dilution, or by abolition of the activity of the sulfhydryl groups, which seems to be brought about by its combination with iodoacetate, and possibly with malonate. It is well known that thiol compounds react rapidly with halogen acids under physiological conditions.[11, 12] The presence of sulfhydryl groups in the sperm fluid was detected by the characteristic color reaction with nitroprusside. The respiration of sperm, high in lower animals, is greatly diminished in mammals. It is possible that the respiration of mammalian sperm is kept at low levels by the inhibiting action of sulfhydryl compounds; iodoacetate would then increase their respiration.

Conclusion. Iodoacetate and malonate added to sea urchin sperm in concentrations sufficient to produce inhibition of respiration in other animal tissues increased the respiration, the increase being equally effective on the O_2 uptake and the CO_2 output. No change in R.Q. was therefore observed. It is postulated that sea urchin sperm fluid possesses a substance—probably a sulfhydryl derivative—which normally keeps the respiration at a low level. Dilution of this substance or its destruction by addition of iodoacetate or malonate would produce an increase of the whole respiratory process.

---

[11] Hellstroem, N., *Z. physik. Chem. Abt. A*, 1931, **157**, 242.

[12] Dickens, F., *Biochem. J.*, 1933, **27**, 1141.

13392

Distribution of Diastase in Serum, Plasma, Whole Blood and Red Blood Cells.*

ADOLPH BERNHARD AND MILTON J. ROSEN.

From the Achelis Laboratory of the Lenox Hill Hospital, New York, N.Y.

The development of an accurate and simplified procedure for the determination of blood diastase has given a new impetus to the study of the diastatic activity of human blood. There is considerable literature concerning diastatic activity of the blood, but it contains no record of any intensive studies on the distribution of diastase in human blood. In view of this it was thought worth while to present the results of our studies in diastase distribution.

Procedures. Blood specimens were obtained by venepuncture, in most cases before breakfast. Whole blood was transferred to a Sanford-Magath hematocrit tube containing either dried sodium oxalate, or purified heparin. The type of anticoagulant used had no effect upon the diastatic activity. The tube containing the well-mixed blood specimen was centrifuged at 3,000 rpm for 30 minutes. The volume of packed red cells was recorded and the red cell volume calculated; the supernatant plasma was used for the determination of diastase activity. Serum was obtained from an additional sample of blood taken simultaneously.

The method employed for the determination of the diastase was essentially that of Somogyi,[1] with the exception that the Benedict[2] 1931 copper method for the estimation of sugar in the tungstic acid filtrates was used. All of our determinations were made on 0.5 ml of serum, plasma, whole blood and red cells. In one subject, No. 187741, several estimations were made at intervals after the patient had undergone treatment.

Discussion. The distribution of diastase between serum, plasma, whole blood and red cells was studied in 26 subjects, see Table I. There was a wide range of diastase values in the cases selected, varying in the serum between 39 and 204. It will be observed that the diastase of the plasma tends to approach that of the serum, although in general it is lower. In 17 instances the plasma value was lower, in 3 exactly alike, and in 6 somewhat higher. The diastase of whole blood is considerably lower than either the serum or plasma.

---

* This work was aided by a contribution from Dr. A. J. Abeloff.

[1] Somogyi, M., *J. Biol. Chem.*, 1938, **125**, 399.

[2] Benedict, S. R., *J. Biol. Chem.*, 1931, **92**, 141.

In our opinion the diastase values of whole blood are too low to be of any diagnostic value. The red cells contain no diastase, the values obtained being between 0 and 13, which are well within the experimental error of the procedure used. The values are independent of the red cell volume. That the red cells contain no amylase has also been reported by Morse[3] in experiments on dogs.

If we multiply the plasma diastase by a factor (1 minus the red cell volume, expressed as a decimal) we obtain the value of the corresponding whole blood diastase. This checks rather well with the actual results of the whole blood estimations, and furnishes further corroboration of our observation that the red cells contain no diastase.

Summary. Distribution studies of diastase in serum, plasma, whole blood and red blood cells from the same specimen of human blood were made in 26 subjects. It was shown that the plasma values approach those of the serum, while the whole blood values are considerably lower. The red cells contain no diastase.

TABLE I.
Distribution of Diastase in Human Blood.

History No.	Serum	Plasma	Whole blood	Red cells	Red cell volume, %
187741	84	61	38	9	32
”	71	80	43	0	43
”	87	90	45	3	41
187527	68	76	36	0	43
72512	48	47	32	4	40
80100	27	23	10	8	19
184694	99	83	47	4	40
187878	74	61	15	2	—
187558	64	59	37	4	42
169972	88	78	57	13	37
—	72	50	16	7	47
180516	70	70	46	1	45
187982	62	54	38	4	37
125164	39	36	20	4	40
154578	43	35	24	3	45
187458	73	75	42	4	46
97582	89	89	53	7	—
80619	204	159	91	6	30
188213	63	69	41	4	49
80975	103	80	47	2	39
186834	44	39	18	1	31
170867	61	61	36	1	28
81157	104	90	46	1	37
42155	75	86	65	7	48
97436	69	82	45	2	42
188409	115	111	60	0	47
60100	154	127	59	3	32
57655	121	109	92	1	13

---

[3] Morse, W., *Proc. Am. J. Biol. Chem.*, 1923, **55**, 27.

13393

Decreased Carbohydrate Appetite of Adrenalectomized Rats.*

CURT P. RICHTER.

From the Psychobiological Laboratory, Phipps Psychiatric Clinic, Johns Hopkins Hospital.

In previous experiments in the self-selection of foods it was reported that adrenalectomized rats had an increased appetite for a 3% solution of sodium chloride and tap water and a decreased appetite for a 40% dextrose solution, but no change in appetite for the stock diet (Richter[1]). Although the results seemed to prove quite conclusively that the adrenalectomized rats had a decreased appetite for the carbohydrate (dextrose), the possibility remained that the rats may have taken less of the dextrose solution because of a markedly increased thirst for plain water. A definite answer to this question was obtained from experiments in which the dextrose was offered in solid form.

In these experiments the rats were kept separately in cages which contained one cup for the saltless stock diet and one for the dextrose and a graduated inverted 100 cc bottle for a 3% solution of sodium chloride and another for tap water. Readings were made daily of the intake from each container and weekly of body weight. Records were taken for 20 days before adrenalectomy and 70 days postoperatively. During the last 30 days the rats were treated with daily injections of desoxycorticosterone (Percorten).

Table I summarizes the results of the observations made on 5 rats. The average daily sodium chloride intake increased from 2.1 cc for the 10-day period immediately preceding adrenalectomy to 12.4 cc for the 30- to 40-day postoperative period; water intake increased from 18.9 cc to 23.0 cc (average daily intake of fluids corrected for evaporation of 1 cc); dextrose intake decreased from 5.8 g to 2.3 g. All 5 rats showed these changes. The intake of stock food increased in 3 rats and decreased in 2 rats.

Daily injections of Percorten (0.1 cc) started on the 40th postoperative day caused a prompt reversal of the appetites. The average sodium chloride intake decreased from 12.4 cc to 1.5 cc, approximately the preoperative level. Water intake decreased from an average of 23.0 cc to 17.4 cc; the average dextrose intake increased

---

* Dr. Ernst Oppenheimer of Ciba Pharmaceutical Products, Inc., Summit, New Jersey, kindly supplied the Percorten for these experiments.

[1] Richter, Curt P., *Endocrinology*, 1941, **29**, 115.

TABLE I.
Dietary Selections of 5 Rats Before and After Adrenalectomy and During Percorten Treatment.

			Sodium chloride, 3%		
Rat No.	Age at operation	Sex	10-day period before operation, cc	30-40-day period after adrenalectomy, cc	Last 10-day period on Percorten, cc
1	155	♂	1.3	10.9	1.0
2	156	♂	2.4	11.7	0.5
3	156	♂	5.0	16.8	3.1
4	176	♂	1.2	8.6	1.2
5	160	♂	0.6	14.2	1.5
Avg	161		2.1	12.4	1.5
			Water		
			cc	cc	cc
1			20.0	22.4	16.4
2			15.0	21.1	19.8
3			16.3	25.0	17.3
4			19.8	20.7	13.5
5			23.2	25.7	20.2
Avg			18.9	23.0	17.4
			Dextrose		
			g	g	g
1			4.4	1.8	4.7
2			8.4	3.0	5.0
3			5.8	1.8	4.6
4			4.7	1.8	4.8
5			5.5	3.3	3.2
Avg			5.8	2.3	4.5
			Stock diet without added sodium chloride		
			g	g	g
1			7.3	10.4	10.7
2			7.5	10.8	9.8
3			9.7	10.6	13.7
4			10.0	9.3	9.8
5			12.4	5.9	12.8
Avg			9.4	9.4	11.4

from 2.3 g to 4.5 g. Four rats showed an increase in food intake, 1 a decrease.

Clinically an inverse relationship between salt and sugar appetite has also been noticed in a variety of conditions, most common of which is diabetes (Wilder[2]).

---

[2] Wilder, Russell M., *Clinical Diabetes Mellitus and Hyperinsulinism*, Philadelphia, W. B. Saunders Company, 1940.

Summary. 1. Five rats were given access to a 3% solution of sodium chloride, tap water, dry dextrose, and the saltless stock diet in 4 separate containers. 2. After adrenalectomy the rats greatly increased their intake of sodium chloride, decreased their intake of dextrose, but continued to eat about the same amount of saltless stock diet as before. They showed a small increase in water intake. 3. Daily treatment with desoxycorticosterone decreased the intake of sodium chloride solution and tap water to the normal levels and increased the dextrose intake nearly to the normal level. 4. Thus it was shown that adrenalectomized rats have a definitely decreased appetite for dextrose, as well as an increased appetite for sodium chloride.

13394

Armstrong Mouse Adapted Poliomyelitis Virus: Effect of pH of Inoculum, and of "Enteric Organism Filtrate."*

William McDowell Hammon and Ernest M. Izumi. (Introduced by K. F. Meyer.)

From the George Williams Hooper Foundation, University of California, San Francisco, California.

pH of the Inoculum. In reviewing the literature on experimental poliomyelitis, no reports have been found on the optimum pH of the inoculum for monkeys or other susceptible animals. Recently, Howitt noted that poliomyelitis virus suspended in broth of acid reaction appeared to accelerate the action of strains of poliomyelitis virus which were of low pathogenicity for monkeys.[1] At her suggestion, since we were working with the Armstrong adapted virus† in mice, experiments were made to determine definitely the possible effect of the pH of the medium in which the virus was suspended. By employing these inexpensive animals large numbers could be used and more conclusive results be obtained than by using monkeys.

Method. Virus suspensions were prepared by grinding a number of brain stems and cords from dying mice with alundum and saline to make a 20% suspension by weight. This was either employed immediately or in some cases was frozen in ampoules and stored

---

* Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

[1] Howitt, B., personal communication.

† The virus was obtained originally from Dr. C. Armstrong.

at –76°C as a stock suspension. The titer of this frozen virus was found to remain constant over a period of months.

Buffer solutions of pH 4.0, 5.0, 6.0 and 7.0 were prepared as follows:

pH 7.0—6.0 cc of M/15 $Na_2HPO_4 . 12H_2O$ and 4.0 cc M/15 KH_2PO_4 per liter of normal saline.

pH 6.0—1.0 cc of M/15 $Na_2HPO_4 . 12H_2O$ and 9.0 cc M/15 KH_2PO_4 per liter of normal saline.

pH 5.0—10.0 cc of N/10 CH_3COOH and 7.1 cc N/10 NaOH (both N/10 solutions in normal saline).

pH 4.0—10.0 cc of N/10 CH_3COOH and 2.0 cc N/10 NaOH (both N/10 solutions in normal saline).

These buffer solutions were autoclaved at 15 lb pressure for 20 minutes, then the pH was checked and any necessary adjustments made with sterile reagents.

Dilutions of virus were prepared by adding the required amount of buffer solution to the stock virus suspension. When low dilutions were required the pH of the final suspension was tested and correction made by adding 10% acetic acid.

When the pH 4.0 or pH 5.0 buffer was added to the virus suspension precipitation regularly occurred. Before inoculation resuspension was effected and inoculation performed quickly before separation occurred in the syringe. All inoculations were made intracerebrally under light ether anesthesia, employing a dose of 0.03 cc. A highly susceptible strain of Rockefeller Swiss mice‡ was used throughout. Other strains of Swiss mice were found to be unsatisfactory due to low susceptibility and irregularity of response. Mice were observed for 21 days and the time of paralysis and of death recorded. Those dying within 24 hours of inoculation were not included in the tabulation of results. In calculating the survival time for each group the survivors were considered as having lived only 22 days. In repeated titrations of virus, where groups of 12 or more mice were employed for each dilution, we found that within a relatively wide range of virus dilutions the mean length of survival, computed in the above manner is in most instances a satisfactory index of the amount of virus employed.

Experiments 1 and 2. Table I shows the combined results of 2 experiments in which groups of 6 mice were inoculated with virus suspensions in dilutions of 1:512 and 1:1024 in buffered saline of pH 4.0, 5.0, 6.0, and 7.0. Practically all mice died except at pH 7.0, where 4 of 18 survived. The mean length of survival is shown for

---

‡ Carsworth Farms, Inc., New City, N.Y.

TABLE I.
Experiments 1 and 2—Effect of Buffered Saline pH 4.0, 5.0, 6.0 and 7.0 and of Enteric Filtrate pH 4.5 on Mortality and Mean Survival Time of Mice.

		Dilutions			
		1:512		1:1,024	
Menstruum	pH	Mortality ratio	Mean days survival	Mortality ratio	Mean days survival
Buffered saline	4.0	12/12	7.6	12/12	9.1
Enteric Filtrate	*4.5*	*12/12*	*10.6*	*9/10*	*9.9*
Buffered Saline	5.0	12/12	12.1	6/6	13.3
” ”	6.0	12/12	13.5	5/6	15.3
” ”	7.0	9/12	14.6	5/6	18.5

each group. In groups of 6, certain irregularities may occur, but it is apparent from the table with the combined results of 2 experiments that the pH 4.0 suspension killed more rapidly than did any of the others and that there is a progressively increasing mean length of survival with increase of pH.

Experiment 3. In this experiment (Table II) a frozen stock virus was used, in dilutions of 1:500, 1:2,000, 1:8,000, 1:32,000, and 1:128,000. A buffer solution of pH 4.0 and one of pH 7.0 were used to prepare the dilutions and 8 mice inoculated with each suspension. In the lower dilutions where all mice died, the pH 4.0 suspension killed the mice in a shorter period of time. At a dilution of 1:8,000 of the pH 4.0 saline, 7 of 8 mice died, and 1 at pH 7.0. At dilutions of 1:32,000 and 1:128,000 of the pH 4.0 suspensions 3 and 2 mice died respectively, and none at the higher pH value.

TABLE II.
Experiments 3, 4 and 5—Effect of Buffered Saline and of Enteric Filtrate at Various Hydrogen Ion Concentrations on Mortality and Mean Survival Time.

		Dilutions									
		1:500		1:2,000		1:8,000		1:32,000		1:128,000	
Menstruum	pH	Mortality ratio	Mean days survival	Mortality ratio	Mean days survival	Mortality ratio	Mean days survival	Mortality ratio	Mean days survival	Mortality ratio	Mean days survival
Exp. 3											
Buffered Saline	4.0	8/8	6.7	8/8	7.5	7/8	14.5	3/8	20.1	2/8	21.1
Enteric Filtrate	*5.0*	*8/8*	*7.2*	*7/7*	*9.7*	*3/8*	*19.7*	*0/8*	—	*0/8*	—
Buffered saline	7.0	6/6	12.0	8/8	9.2	1/8	21.1	0/8	—	0/8	—
Exp. 4											
Enteric Filtrate	4.5	—	—	8/10	11.3	6/10	14.9	—	—	—	—
” ”	7.6	—	—	6/10	16.9	3/10	19.2	—	—	—	—
Exp. 5											
Enteric Filtrate	4.5	—	—	8/9	12.5	7/10	14.4	—	—	—	—
Buffered Saline	4.5	—	—	9/10	12.3	5/7	17.7	—	—	—	—
Enteric Filtrate	7.6	9/9	12.9	5/8	20.5	—	—	—	—	—	—
Buffered Saline	7.6	11/12	13.5	6/10	16.7	—	—	—	—	—	—

Experiments with Monkeys. Too few monkeys have been used to draw definite conclusions, but work to date with the Armstrong mouse adapted virus (160th mouse passage) in *Macacus philippinensis* monkeys suggests that a virus suspension of pH 4.0 is more effective than one of pH 7.0. Monkeys undergo a fulminating, fatal infection with rapidly progressing flaccid paralysis and with a short incubation period, when inoculated intracerebrally with the acid virus suspension. The virus was recovered readily in mice by inoculation of the monkey cord.

"ENTERIC ORGANISM FILTRATE." Toomey[2, 3] reported that when typhoid-paratyphoid toxic broth filtrate was mixed with a suspension of poliomyelitis virus and inoculated intracerebrally in monkeys a shortened incubation period and a more fulminating disease process resulted. Later by means of virus suspended in a similar filtrate, Toomey and Takacs[4, 5] were able to adapt to the cotton rat Flexner's "M. V." strain and 3 other strains of poliomyelitis virus.

"Enteric filtrate" was prepared as described by Toomey[2] using strains of typhoid, paratyphoid A, paratyphoid B, and several strains of organisms of the colon group obtained from monkey stools. The pH was found to be 4.5 before any adjustment was made. Although Toomey adjusted the pH to approximate neutrality in his work with monkeys[3] and in most of his work with cotton rats,[6] our tests were made in a similar manner to those with buffered saline as described above, using the enteric filtrate at various hydrogen ion concentrations.

Tables I and II (Experiments 1, 2, and 3) present the results of tests made with suspensions of virus in enteric filtrate at a low pH, together with similar virus suspensions in buffered saline. No advantage was noted for the enteric filtrate other than that attributable to the low pH.

Experiment 4. This experiment was planned to determine if the enteric filtrate was more effective at a low pH than when adjusted to pH 7.6. Two dilutions, 1:2,000 and 1:8,000, were used at pH 4.5 and pH 7.6 and 10 mice inoculated with each suspension. Table II shows that the pH of 4.5 was more effective than that of 7.6, as had been the case when saline was used.

---

[2] Toomey, J. A., *J. Inf. Dis.*, 1934, **54**, 74.

[3] Toomey, J. A., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1015.

[4] Toomey, J. A., and Takacs, W. S., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 364.

[5] Toomey, J. A., and Takacs, W. S., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 22.

[6] Personal communication.

Experiment 5. To determine whether enteric filtrate was endowed with any toxic property or accelerating effect that would render it more effective than a buffered saline solution at the same pH, groups of mice were inoculated with virus suspended in each menstruum at pH 4.5 and 7.6, employing 2 suitable dilutions at each pH. Table II shows the results obtained. No significant difference is noted between the effect of enteric filtrate and of saline at either a low pH or neutrality, in fatality rate or mean survival time. Again, the effect of a low pH is clearly demonstrated.

Summary. It has been demonstrated that in the mouse, employing the Armstrong mouse adapted Lansing strain of virus, a definite quantity of virus can be rendered more effective in producing infection by suspending it in a menstruum of low pH. By progressively decreasing the pH from 7.0 to 4.0 the effectiveness of the virus was increased in a step-like manner. The virus suspension at pH 4.0 appeared to be as effective as from 4 to 16 times as much virus at pH 7.0. At present the mechanism is not understood, although numerous conjectures might be made both in respect to local and possible systemic effects. Experiments to investigate the mechanism are in progress.

We have not compared the effect of neutralized enteric filtrate to that of neutral saline in monkeys and cotton rats, as was done by Toomey and by Toomey and Takacs, since these animals were not readily available in sufficiently large numbers to permit clear-cut evaluation of results. However, in the mouse, the effect of the Armstrong virus was not altered by combining it with enteric filtrate. At either a low pH or at pH 7.6 buffered saline has been shown to produce results of an entirely comparable nature.

13395

Influence of Age on Serosal Tumoral Reaction of the Guinea Pig Towards Estrogens.*

Alexander Lipschütz and Luis Vargas, Jr.

From the Department of Experimental Medicine, National Health Service of the Republic of Chile, Santiago.

Uterine and extragenital peritoneal fibroids have been elicited in almost all castrate adult female guinea pigs given sufficient quantities of estradiol benzoate subcutaneously during a 4-months period.[1] This fact raised the question whether or not *young* animals would react with production of abdominal fibroids as *adult* animals do. Uterine fibromyoma occurs in women before puberty rarely if at all; fibromatogenesis in women is seemingly intimately connected with ovarian endocrine activity. However, in experimental fibromatogenesis elicited by estrogens, extragonadal factors also may be in play, as is demonstrated by the fact that the conjunctive tumoral reaction towards estrogens is less pronounced in the castrate *male* guinea pig than in the castrate *female*.[2] Accordingly, the question of the influence of age irrespective of the gonad on the fibromatogenic reaction of castrate female guinea pigs towards estrogens is of considerable interest. Nelson,[3] who was the first to report uterine fibroids elicited by estrogens, induced them in guinea pigs treated from birth. However, he does not refer to extragenital fibroids, and treatment in his experiments was, with but one or two exceptions, prolonged for 8 to 10 months, so that no judgment is possible as to the moment when these fibroids originated.

In our present work† comparison was made between 2 groups of castrate females *very different in body weight* since exact knowledge

---

* This work was aided by a grant from the Jane Coffin Childs Memorial Fund for Medical Research, and from the Rockefeller Foundation. Grants administered by Professor A. Lipschütz. The estrogens were generously supplied by Dr. Carl Miescher of Messers Ciba in Basel.

[1] Lipschütz, A., and Iglesias, R., *C. R. Soc. Biol.* (Paris), 1938, **129**, 519; Iglesias, R., *Pub. Med. Exp.* (Chile), 1938, No. 1; Lipschütz, A., and Vargas, L., Jr., *Cancer Research*, 1941, **1**, 236.

[2] Koref, O., Lipschütz, A., and Vargas, L., Jr., *C. R. Soc. Biol.* (Paris), 1939, **130**, 303; Jedlicky, A., Lipschütz, A., and Vargas, L., Jr., *C. R. Soc. Biol.* (Paris), 1939, **130**, 1466; Lipschütz, A., Vargas, L., Jr., and Palma, J., *Cancer Research*, 1941, **1**, 575.

[3] Nelson, W. O., *Anat. Rec.*, 1937, **68**, 99; *Endocrinology*, 1939, **24**, 50.

† Thanks are due to Dr. Armida Zepeda of the Departamento de Sanidad Escolar, National Health Service, for technical help in the course of this work.

of age was not available. "Aged" animals weighing 700 to 1050 g at beginning of treatment were compared with "young" animals weighing between 180 and 245 g. Subcutaneous injections of 80 γ of estradiol given as monobenzoate in oily solution were administered 3 times a week to all animals. Treatment was prolonged for only 2½ to 3 months so as to completely avoid the possibility of a young animal approaching the limit of body weight range of the "aged" group. Part of the young animals even received a smaller number of injections than the aged ones. For a quantitative evaluation of the tumoral effect a system of classification was used as formerly described.[4] Results are given in Table I. Eight of 11 animals of

TABLE I.

21 Castrate Female Guinea Pigs of High (700 to 1050 g) and Low (180 to 245 g) Body Weight Receiving Subcutaneous Injections of 80 γ of Estradiol (Monobenzoate) Three Times a Week.

					Tumoral effect: class					
Series and No.	Initial wt, g	Final wt, g	No. inj.	Days of exper.	Subserous or parametrial uterine tumors	"Apical"	Digest. tract and abdom. wall	Spleen	Total tumoral effect	
XV										
10	700	685	38	88	0	0.5 f	0	0	0.5	
7	735	720	37	89	2	0	2	2	6.0	
19	750	700	38	89	0	0	0.5 f	0	0.5	
8	765	790	38	88	1	1	2	2	6.0	
9	790	685	37	89	1	2	1	2	6.0	
11	900	790	38	88	2	3	3	3	11.0	4.4
2	920	795	38	89	0.5 t	0.5 f	2	0.5 s	3.5	
3	950	805	37	88	0	0	0.5 f	0	0.5	
1	960	720	37	87	0	0.5 f	3	1	4.5	
12	980	960	38	88	1	0.5 f	1	0	2.5	
6	1050	870	38	90	0	3	3	2	8.0	
XV										
24	190	390	38	90	2	2	3	0	7.0	
4	200	385	35	91	0	3	2	2	7.0	
20	245	410	39	91	1	2	0.5 f	2	5.5	
IX										
13	180	460	27	75	0	2	1	2	5.0	4.9
16	190	455	30	84	0	3	1	0.5 t	4.5	
20	205	490	27	77	0	2	1	0	3.0	
15	210	450	27	75	0	1	0	0	1.0	
21	220	540	27	77	0	2	0	1	3.5	
17	230	460	27	76	0	0.5 f	0.5 f	0.5 t	1.5	
19	240	370	30	85	3	3	2	3	11.0	

f = fibrous strands only.
t = one or several small tumoral nodules.
s = tumoral seed, *i.e.*, accumulation of minute tumoral nodules.

---

[4] Lipschütz, A., and Vargas, L., Jr., *C. R. Soc. Biol.* (Paris), 1939, **131**, 27; Lipschütz, A., Bellolio, P., Chaume, J., and Vargas, L., Jr., Proc. Soc. Exp. Biol. and Med., 1941, **46**, 164.

high body weight (700 to 1050 g) developed uterine or extragenital fibroids of class 1 or more; the remaining 3 animals showed some form of abdominal fibrous reaction. The average total tumoral effect was 4.4. All animals of low body weight treated for the same length of time as those of high body weight (series XV), gave a conspicuous tumoral reaction. Uterine and extragenital fibroids were present even in 5 of those 7 young animals which were treated for a shorter time (series IX) than the aged animals.

Table I gives full evidence that peritoneal fibroids can be elicited not only in the adult, but also in young females when treatment begins at a body weight of less than 200 g and when necropsy is made before a body weight of 500 g is reached. There was no difference in the frequency of abdominal fibroids in the two groups.

Another question also arises here. The average total tumoral effect in young animals, even when treated for a shorter time, as in series IX, was not less than in adult of aged animals. Does this mean that age has no influence on the *degree* of the fibromatous reaction, *i. e.,* on the size and number of fibroids? The number of "resistant" castrate female guinea pigs, *i. e.,* animals which when treated with estrogens for several months showed no conjunctive reaction, was

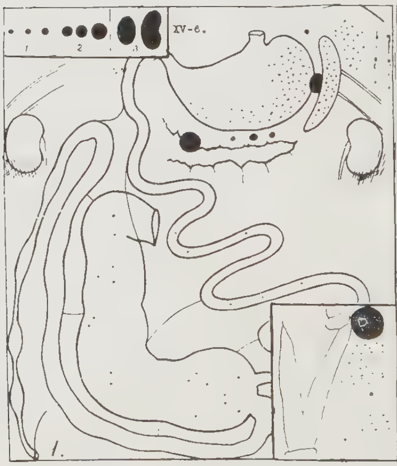


FIG. 1.

Castrate female guinea pig XV, 6. Weight at beginning—1050 g, at end of experiment—870 g. 38 injections of 80 γ of estradiol (benzoate) in the course of 90 days. Large apical tumor of left horn and various extragenital tumors. Tumoral seed at different places. In this case there was also considerable development of fibrous strands at different places; they are mostly omitted in the diagram.

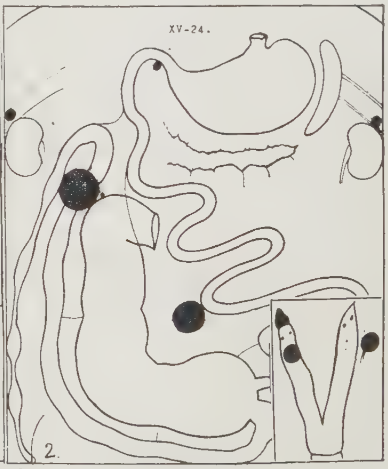


FIG. 2.

Castrate female guinea pig XV, 24. Weight at beginning—190 g, at end of experiment—390 g. 38 injections of 80 γ of estradiol (benzoate) in the course of 90 days. Large uterine and extragenital tumors.

very low in the work of Lipschütz and fellow workers during the last 4 years. On the other hand, there was considerable individual variation as to the degree of the fibromatous reaction, which may range between 0.5 and 12 according to our classification.[4] For this reason too much importance should not be attributed as yet to the coincidence in the average total tumoral effect in animals of high and low body weight.

Summary. Uterine and extragenital fibroids were elicited by subcutaneous administration of estradiol monobenzoate in castrate female guinea pigs weighing no more than 180 to 245 g at the beginning of treatment and autopsied 2½ to 3 months later before they reached a body weight of 500 g. The incidence of abdominal fibroids in these young animals was no less than in a group of adult or aged animals weighing 700 to 1050 g at the beginning of treatment. Whereas this statement referring to the occurrence of experimental abdominal fibroids in young females seems definite, the question remains open whether age may influence the degree of the fibromatous reaction, *i. e.,* size and number of fibroids.

13396

Urinary Excretion of Trichina Antigen in Experimental Trichinosis.*

LOUIS G. WELT. (Introduced by Francis G. Blake.)

From the Section of Preventive Medicine, Yale University School of Medicine, New Haven, Conn.

The elimination by the kidneys of substances derived from bacterial cells has been noted in several human infections such as the urinary excretion of capsular polysaccharide in pneumococcal infections,[1] and the excretion of scarlatinal toxin in scarlet fever.[2] It is probable that this mechanism is operative in a number of bacterial infections although the field has not been adequately explored. The present note describes an example of the same phenomenon in an experimental disease of parasitic origin.

In this study the urinary excretion of an antigenic substance, or

---

* Aided by a grant from the Fluid Research Fund, Yale University School of Medicine.

[1] Dochez, A. R., and Avery, O. T., PROC. SOC. EXP. BIOL. AND MED., 1917, **14**, 126.

[2] Trask, J. D., and Blake, F. G., *J. Exp. Med.,* 1924, **40**, 381.

substances, derived from *Trichinella spiralis* was observed in experimentally infested rhesus monkeys. Infestation was accomplished by feeding by stomach tube approximately one trichina larva per gram of body weight to 2 monkeys *(Macaca mulatta)*. Following infestation the monkeys were observed for 10-12 weeks respectively, for the following items: daily temperature, general behavior and physical phenomena, eosinophilia, skin reaction to trichina antigen, trichina antigen in serum and urine, and trichina antibody in serum.

Methods. Twenty-four-hour samples of urine were collected in metabolism cages at stated intervals (Fig. 1). This urine was dialyzed for 15 hours through cellophane tubing against tap water at icebox temperature to remove excess salts. The urine was then evaporated to dryness in Petri dishes at room temperature. The dried urine sediment was redissolved in about 5 cc sterile saline and centrifuged and the supernatant fluid was saved and used in the precipitin tests. The final volume represented approximately a 10-fold concentration.

Trichina antigen in serum and urine was demonstrated by precipitin reactions using rabbit serum having a high titer of trichina antibody.[†] The trichina antibody in monkey sera was demonstrated by precipitin reactions employing dilutions of trichina antigen[‡] prepared from dried larvae by a method described by Witebsky.[3] The serum-antigen mixtures were incubated at 37°C in tubes 4 mm inside diameter for one hour and then allowed to stand over night in the icebox. Tests for trichina antigen in urine were read at 24 and 48 hours; flocculation first appearing in 48 hours was designated as 1+, and that appearing in 24 hours as 2+. The readings of tests in the cases of serum antigen and antibody were made in 24 hours. Skin tests were performed with 1:500 solution of trichina antigen in the skin of eyelid. At postmortem in each instance trichina larvae were demonstrated in the respective diaphragmatic muscle and both monkeys were found to have widespread tuberculosis, as had been expected.

Results. As the course and results in the infested monkeys were approximately the same in both, one representative chart may suffice to indicate what happened in each animal. The prolonged urinary excretion of trichina antigen over a period of at least 50 to 70 days is

---

[†] I am indebted to Dr. E. Witebsky of the University of Buffalo Medical School for a supply of trichina antiserum.

[‡] I am indebted to the Eli Lilly Drug Company for a supply of dried trichina larvæ.

[3] Witebsky, E., Wels, P., and Heide, A., *J. Bact.*, 1941, **41**, 65.

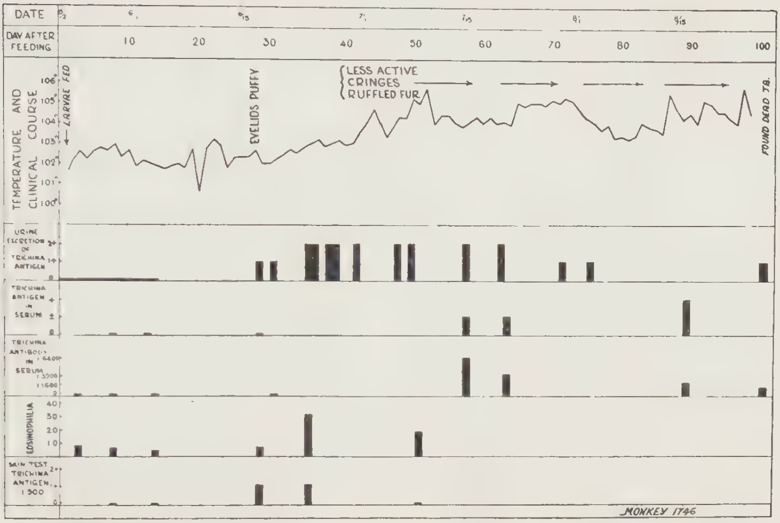


FIG. 1.

Temperature chart, tests for urinary antigen, blood antigen and antibody; eosinophilia and skin tests in one of two monkeys experimentally infested with trichina larvæ.

in some contrast to the eosinophilia and positive skin tests which, occurring early in the disease, were of shorter duration. Some of the fever during the last few weeks of the monkey's life was no doubt due to tuberculosis.

Fig. 1 represents graphically the clinical course and immunological data on one of 2 experimentally trichina infested monkeys and is self-explanatory.

McCoy[4] claimed that the symptoms of fever, edema and eosinophilia did not appear in the monkeys he had infested with trichinae. Our results are contrary to his findings.

Comment. Antigen was present in both blood and urine during early and late stages of a heavy infestation of experimentally induced trichinosis. Moreover, the antigen in the serum existed in a free (or, at least, easily demonstrable) form in the presence of antibody. The continued urinary excretion of the antigen over a period of weeks is noteworthy.

Conclusions. 1. Rhesus monkeys infested with trichina larvae may develop fever, edema, and eosinophilia. 2. Trichina antigen is demonstrable in the serum and urine of trichina infested monkeys over a period of several weeks and at a time when trichina antibody is also demonstrable in the serum of these monkeys.

---

[4] McCoy, O. R., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 85.

13397 P

Mammary Growth in Hypophysectomized Male Mice Receiving Estrogen and Prolactin.*

W. U. Gardner and Abraham White.

From the Departments of Anatomy and Physiological Chemistry, Yale University School of Medicine.

The first experiments revealing the lactogenic activity of hypophyseal extracts were inadequate for the demonstration of mammary growth.[1] Later experiments suggested a proliferation of the mammary glands of immature ovariectomized rabbits receiving hypophyseal extracts.[2] Other investigators detected little mammary growth in animals receiving prolactin.[3, 4] In more recent experiments mammary growth occurred in ovariectomized, in male or in hypophysectomized mice, or in rats following implantation of hypophyseal tissue or injection of hypophyseal extracts.[4, 6, 7, 8] Some investigators associated this mammogenic activity with the protein-components of such extracts,[7, 8] others with the lipid soluble material of pituitary tissue.[6]

Although administration of estrogen results in mammary growth in intact rodents, the mammary response of hypophysectomized animals has been inconsistent.[4, 5] The mammary glands of some hypophysectomized male mice developed slightly when estrogen, and progesterone or desoxycorticosterone were injected. Estrogen alone was least effective and the combinations most effective.[9] The present experiments were concerned with the action of purified lactogenic hormone alone or in combination with estrogen on the mammary rudiments of hypophysectomized male mice.

---

* These investigations have been supported by grants from the Anna Fuller Fund, the Jane Coffin Childs Memorial Fund for Medical Research, the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association, and the Fluid Research Fund of the Yale University School of Medicine.

[1] Stricker, P., and Gruter, F., *Comp. rend. Soc. de biol.*, 1928, **99**, 1978.

[2] Corner, G. W., *Am. J. Physiol.*, 1930, **95**, 43.

[3] Gardner, W. U., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bull.* 196, 1933.

[4] Turner, C. W., ''Mammary Glands,'' Chap. XI, *Sex and Internal Secretions*, E. Allen, C. H. Danforth and E. A. Doisy, Editors, Williams and Wilkins, Baltimore.

[5] Nelson, W. O., *Physiol. Rev.*, 1936, **16**, 488.

[6] Lewis, A. A., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bull.* 310, 1938.

[7] Reese, R. P., and Leonard, S. L., *Endocrinology*, 1941, **29**, 297.

[8] Greep, R. O., and Stavely, H. E., *Endocrinology*, 1941, **29**, 18; Gardner, W. U., and White, A., unpublished.

[9] Gardner, W. U., Proc. Soc. Exp. Biol. and Med., 1940, **45**, 835.

Hybrid male mice from 4 to 8 weeks of age were hypophysectomized using the procedure described by Thomas.[10] The mice were maintained on a diet of Fox Chow and water. Completeness of operation was checked by examination of serial sections of the sella. From 3 to 47 days after operation injections of lactogenic hormone or of the latter together with estrogen[†] were started, the former administered intraperitoneally and the latter subcutaneously on alternate days. On the tenth to thirteenth day the animals were killed and the mammary glands studied from stained and dissected preparations. As the glands of untreated intact, or of hypophysectomized male mice were always extremely rudimentary, only 2 untreated controls were included in this series. They failed to show mammary growth.

Five mice received a commercial preparation of prolactin (SC) which contained 12.5 I.U. per mg. A slight mammary growth occurred in 3 mice. Two of these were caged with animals receiving

TABLE I.
Mammary Responses in Hypophysectomized Mice Receiving Only Prolactin or Prolactin Together with Estrogens.

No. of mice	Treatment*	Period treated (days)	Days post-operative at start	No. complete operations	No. incomplete operations	No. mice showing mammary growth	
						Operations complete	Operations incomplete
3	DPB and SC	12	3	2	1	2	1
1	DPB and AW	10	6	1		1	
2	DPB and SC	11	26	2		2	
3	DPB and SP	9	30	3		3	
3	DPB and SP	9	46	3		3	
3	DPB and SP†	9	47	2	1	2	1
2	SC	12	3	2		2 slight	
2	AW	10	6	2			
2	AW†	10	6	1	1		
3	SC	11	26	2	1	1	
2	SP	9	30	2			
2	SP	9	46	1	1		
2	SP†	9	34	1	1		

Each prolactin preparation was given in a dose of 1 mg daily, except in groups marked † which received ⅓ mg daily.

*SC—A prolactin preparation (Schering Corporation) containing 12.5 I.U. per mg.
AW—A purified prolactin preparation—35 I.U. per mg.‡
SP—A purified prolactin preparation (Schering) containing 35 I.U. per mg.
DPB—Estradiol dipropionate 1 μg every second day.

‡This prolactin preparation was demonstrated to be homogeneous as shown by studies of its solubility, electrophoretic behavior, and sedimentation in the ultracentrifuge (unpublished results.)

---

[10] Thomas, F., *Endocrinology*, 1938, **22**, 99.

[†] The estradiol dipropionate and 2 preparations of prolactin (SC and SP) were generously supplied by the Schering Corporation through the courtesy of Dr. E. Schwenk.

estrogen and may have acquired enough estrogen by contact to elicit some mammary response. A moderately extensive mammary growth occurred in all mice receiving both estrogen and the same preparation of prolactin (Table I).

The rudimentary glands were not stimulated in the 10 operated animals receiving either of the 2 preparations of highly purified prolactin (SP and AW) at either of the 2 different dose levels. The 10 operated mice receiving estrogen in addition to the purified lactogenic preparations showed mammary growth rated as ++ in 8 animals and + in the other two. At the time they were removed the glands were growing rapidly as indicated by the enlarged end buds containing numerous mitotic figures. The extent of mammary proliferation averaged somewhat less than that obtained in unoperated mice receiving estrogen, and was comparable to that induced in operated mice receiving estrogen and progesterone.[9]

The failure of mammary growth to occur in hypophysectomized rats receiving lactogenic hormone has been demonstrated.[11] Mammary growth likewise failed to occur in hypophysectomized male mice when given the purified preparation of lactogenic hormone used in this investigation. The mammary growth occurring when estrogen was administered together with the purified lactogenic hormone indicates that prolactin may either sensitize the mammary tissue of hypophysectomized mice to estrogen or improve the general condition of these animals so that mammary proliferation may occur.

Summary. The mammary rudiments grew in hypophysectomized male mice receiving either of 2 purified preparations of prolactin and estrogen. The injection of these preparations of prolactin alone was not followed by mammary growth.

13398

Some Effects of Thyrotropic Hormone on the Pregnant Rat.

CHARLES E. TOBIN. (Introduced by Karl E. Mason.)

From the Department of Anatomy, The University of Rochester, School of Medicine and Dentistry.

Grumbrecht and Loeser[1] reported death and maceration of the embryos in rats treated with thyrotropic hormone, whereas the ovaries of their animals were found to be enlarged but otherwise normal. Rats are known to be relatively unresponsive to the thyrotropic hormone and the available preparations of this hormone apparently contain luteinizing as well as thyroid-stimulating principles.[2-5] The following experiments, therefore, were undertaken on pregnant rats to determine whether (1) there would be death and resorption of the embryos; (2) luteinization of the unruptured Graafian follicles; and (3) histological evidence of stimulation of the maternal and embryonic thyroids.

---

[11] Evans, H. M., Simpson, M. E., and Lyons, W. R., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 586.

Insemination of virgin albino rats (inbred Wistar strain) was determined by the vaginal smear method and on the 10th day of pregnancy the number of implantation sites ascertained by laparotomy. Daily intraperitoneal injections of thyrotropic hormone* were begun the next day and given in the amounts and for the periods indicated in Table I. The animals were killed on the 16th day and the number of living and dead embryos counted. One ovary, 1 lobe of the thyroid, and a portion of the embryonic sites were fixed in Bouin's solution, sectioned and stained with hematoxylin and eosin.

Results. 1. The percentage of living embryos was inversely proportional to the duration, rather than the size, of the dose; for a smaller number of living embryos was observed when a 0.5 cc dose was distributed over a period of 5 days than if a similar dose was given as a single injection† (Table I). 2. With the exception of the animal injected with 0.1 cc dose, whose ovarian histology was normal, the ovaries of all the other animals contained 3 to 20 unruptured Graafian follicles in the process of luteinization (pseudolutein bodies). No correlation was found between the number of pseudolutein bodies and the amount of hormone administered. 3. The maternal-thyroids showed histological evidence of stimulation when the animals were treated for 2 days or more; no response of the

---

[1] Grumbrecht, P., and Loeser, A., *Klin. Woch.*, 1938, **17**, 233.

[2] Loeb, L., and Friedman, H., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 172.

[3] Starr, P., Patton, H., and Bruner, R. G., *Medical Papers Dedicated to Dr. H. A. Christian*, 1936, 329.

[4] Stephens, D. J., and Allen, W. M., *Endocrinol.*, 1941, **28**, 580.

[5] Tobin, C. E., in press.

* The thyrotropic hormone (Antuitrin T), which was assayed to contain 50 to 100 guinea pig units per cc, was generously supplied by Parke, Davis & Company.

† The average number of implantation sites was 10.2 (5-13) on the 10th day. Since the average litter size of our colony is 8 young, a limited number of embryonic deaths and resorptions normally occur before delivery. K. E. Mason (personal communication) found in 50 stock animals, which were autopsied on the 16th day of pregnancy, that an average of 89% of the implantation sites contained live embryos.

TABLE I.
Pregnant Rats Treated with Thyrotropic Hormone.*

No. of animals		Dosage			Avg % embryos alive 16th day	Implantation sites and embryos alive in each animal
		Total cc	Single inj., cc	Days		
1		.1	.1	1	90	*10* (9)†
5	2	.2	.1	2	100	*13* (13); *11* (11)
	3	.2	.2	1	87	*12* (10); *8* (7); *12* (11)
10	3	.5	.5	1	91	*12* (11); *9* (8); *12* (11)
	7	.5	.1	5	17	*12* (0); *10* (1); *12* (0); *10* (2); *10* (5); *5* (1); *10* (3)
4		1.0	.2	5	6	*7* (0); *10* (1); *8* (0); *6* (1)
4		0	0	0	89	*10* (8); *7* (6); *12* (11); *10* (10)

†Number in italics—number of implantation sites on 10th day.
Number in parenthesis—Number of embryos alive on 16th day.

thyroid could be elicited by injections given over a shorter period. The thyroids of the embryos, which had not undergone autolysis, did not show any histological signs of stimulation even though the maternal-thyroids were stimulated.

The embryonic deaths are attributed to the secretion from the stimulated maternal-thyroids rather than the abnormal luteinization of the ovaries. The incidence of embryonic death prior to the 16th day was much greater in those animals whose thyroids showed histological evidence of stimulation than in those not so stimulated, and bore no constant relation to the number of pseudolutein bodies present.

As a control for hyperthyroidism produced by the thyrotropic hormone injections, 10 animals (studied as in the previous experiment) were given daily intraperitoneal injections of crystalline thyroxin‡ in the dosage and for the periods shown in Table II. Animals Nos. 1-3 were killed on the 13th day; the others on the 16th day. In only one case (No. 5) was there an abnormal number of dead embryos. This treatment did not alter the histological structure of the thyroids from that usually found during pregnancy. The ovaries, however, contained many atretic follicles but no pseudolutein bodies.

---

‡ Crystalline thyroxin (E. R. Squibb & Sons) was injected in the form of 1 mg per cc solution of N/100th NaOH. First injection was given on the 11th day of pregnancy.

TABLE II.
Pregnant Rats Treated with Thyroxin.[‡]

	Dosage				
Animal No.	Total mg	Single inj., mg	Days	% embryos alive 16th day	Implantation sites and embryos alive in each animal
1	.1	.1	1	100	*9* (9)[†]
2	.4	.2	2	92	*13* (12)
3	.6	.2	3	100	*10* (10)
4	.8	.2	4	91	*11* (10)
5	1.0	.2	5	60	*10* (6)
6	1.0	.2	5	90	*10* (9)
7	1.0	.2	5	100	*6* (6)
8	1.0	.2	5	89	*9* (8)
9	1.2	.4 .2	1 4	90	*10* (9)
10	1.4	.4 .2	2 3	100	*12* (12)

[†]Number in italics—number of implantation sites on 10th day.
Number in parenthesis—number of embryos alive on 16th day.

The dose of thyroxin used in this experiment was not large enough to produce any marked effects in the animals and since it was not given during the last quarter of pregnancy the survival of the embryos was not affected. Bodansky and Duff[6] found that pregnant rats treated with 1 mg of thyroxin daily during the last 10-12 days of pregnancy showed only a moderate loss in body weight and delivered the same number of young, but a higher percentage of still births, than controls. Since the period of gestation was prolonged,[6] crystalline thyroxin must interfere with the birth mechanism and cause embryonic deaths only during the latter part of gestation.

Conclusion. Rats injected with thyrotropic hormone, between the 10th and 16th days of pregnancy, showed a high percentage of dead embryos, stimulation of the maternal-thyroid and luteinization of unruptured Graafian follicles. The embryonic deaths are attributed to secretion from the stimulated maternal-thyroid. Injections of crystalline thyroxin produced no such effects.

---

[6] Bodansky, M., and Duff, V. B., *Endocrinol.*, 1936, **20**, 537.

13399 P

Effect of Electric Convulsive Therapy on Memory.

JOSEPH ZUBIN AND S. EUGENE BARRERA. (Introduced by C. Landis.)

From the Departments of Psychology and of Psychiatry, New York State Psychiatric Institute and Hospital

Electric shock therapy is one of the therapies that is being used currently in the treatment of mental disease. The technic utilized at the New York State Psychiatric Institute has been described[1] and the exact procedure together with the clinically observed sequelae were carefully noted. One of the most striking psychological concomitants of this treatment is the characteristic impairment of the patient's memory.

In order to obtain objective evidence of the degree of memory impairment, it was determined to teach 10 patients a series of tasks before treatment and note the influence of the treatment on the retention of this material.

The materials used in this experiment were paired word associates composed of common household commodities like sugar or coffee paired with pseudo-brand names consisting of two-syllabled nonsense material.

A week before treatment was begun the patient was given a control series consisting of a learning session in the morning followed by a retention test in the afternoon. The criterion of success was 2 consecutive correct repetitions. During the shock week the same procedure was followed on the shock days (Monday, Wednesday and Friday) as well as on the alternate non-shock days. At the end of the course of treatment, several of the patients were given a post-treatment series of tests.

Results. The effect of shock on memory was analyzed in accordance with the 3 methods available for testing retention: relearning, recall and recognition. The data for each patient were analyzed separately and after the consistency of the individual results was established they were combined. When no shock intervenes between learning and relearning, the number of trials saved is significant when compared to its standard error. However, when a shock is interpolated between learning and relearning, there is no significant saving, and indeed there is a slight, but non-significant loss. It should be noted, however, that learning ability is no poorer after shock than before shock.

---

[1] Kalinowsky, L., and Barrera, S. Eugene, *Psychiat. Quart.*, 1940, **14**, 719.

The first trial in each relearning series was regarded as the recall trial, and the number of correctly recalled items in that trial constituted the recall score. The recall after shock was significantly less than the recall after the control and non-shock periods. It is interesting to note that the highest recall is shown in the post-treatment testing.

In addition to the evidence of memory impairment given by the saving and recall methods, the recognition method also yielded an index of memory loss. The afternoon before each shock day a series of paired associates was taught and in the morning immediately before shock, another series of paired associates was taught in which the commodities were identical with those taught the previous night. There were thus, two associates to each commodity, a "remote" one, learned the night before and a "recent" one, learned immediately before shock. After shock, when the patient had regained his composure, a recognition test was given to the patient. This test consisted of the commodity name followed by 4 choices, 2 of which were the learned brand names.

Under control conditions, the patients were able to recognize correctly both paired associates ("recent" and "remote") nearly 94% of the time. Under shock conditions, this percent dropped to 81.5. This represents a drop of 12.5 points which is found to be statistically significant. It should be noted, however, that shock interferes only slightly with recognition, for the post-shock recognition is 87% as strong as the post-control recognition. The recognition under non-shock conditions is very similar to the performance under shock conditions. It should be noted that even under shock conditions the performance is significantly greater than chance. This holds true not only of the group results, but also of the results for each individual.

It is interesting to note that under control conditions "recent" learning is stronger than "remote" learning for, in 5.8% of the items the recent associate was recognized while the remote associate failed to be recognized. Under shock conditions, the differential between "recent" and "remote" learning disappears, as shown by the fact that the proportions in the "recent only" and "remote only" categories are about the same. Apparently the shock affects the learning acquired immediately before shock more than the learning acquired less recently before shock.

13400

Rate of Disappearance of Subcutaneous Vegetable Oil in Normal and Castrate Mice.*

JOSEPH C. TURNER AND BARBARA MULLIKEN. (Introduced by W. W. Palmer.)

From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York City.

Among the problems of fat metabolism so-called simple obesity has long been of interest to physicians. One point of view taken with regard to this condition considers it as merely another example of the operation of the law of conservation of energy. This attitude is, of course, unassailable, since it must be true that there is a disparity between expenditure of energy and food intake, if fat is to be stored in the subcutaneous tissues. At the same time, however, the phenomenon of obesity would appear to contain relationships of a biological nature which are less well defined. In the human subject, for example, obesity occurs with great frequency following certain disturbances of endocrine function, such as menopause, castration, or pituitary disease. No doubt in each instance there is a displacement of the equilibrium between caloric intake and output due to lowering of the total metabolism. But that this is the whole story is not so clear. The rate of deposition or utilization of depot fat and the type of mechanism called upon for its ultimate disposal both may be influenced by factors that are as yet poorly understood. The clinical association of obesity with abnormal endocrine function lends interest to this report of an observation which seems to indicate that even in normal animals there is a relation between sex and the rate of disappearance of an oil injected parenterally.

In the course of an experiment designed to study the effect of o-amino-azotoluol on the liver, the dye was dissolved in corn-oil ("Mazola") and injected subcutaneously into the backs of mice. The mice were of the closely inbred Bagg albino (C) strain and were all about 4 months old when first treated. They were 60 in number, 30 males and 30 virgin females, and were maintained on a diet of stock pellets which provides all of the necessary vitamins. As far as is known, they were normal animals. Each mouse received regularly each week for several months 0.1 ml of oil containing 2.5 mg of azotoluol. After about 3 months it was noted that there was a striking difference in the appearance of the males as compared

---

* This work was carried out in part under a fellowship grant from the Commonwealth Fund.

with the females. The latter showed large humps on their backs representing encapsulated oil. The males, however, had failed to develop such swellings. This curious dissimilarity was quite unexpected, because the assumption had been made on the basis of general experience that such oils are physiologically almost inert and it seemed that these particular males had disposed of a surprisingly large quantity. Moreover, the difference was shown not only by a few animals but characterized virtually every one, so that it was possible by a glance at the back of any mouse to judge its sex with remarkable accuracy.

At first it was thought possible that this variation in behavior was dependent upon the presence of the biologically active carcinogen, and therefore the experiment was repeated using Mazola alone. Twenty male and 12 female Bagg albino mice about 3½ months of age were given 5 injections of 0/1 ml of oil at intervals of several days over a period of 3 weeks. One week after the last injection, 10 of the 12 females showed characteristic swellings of moderate size. Of the 20 males, only 6 presented any nodules and 4 of these were very small, while 14 had no visible or palpable collections of oil. At autopsy, direct examination of the involved areas bore out the preliminary impression that the amount of oil remaining in the females was significantly larger. Three weeks after the last injection, 4 males and 3 females that had been spared were examined again. The males, viewed from without, were negative but upon dissection showed very small droplets or blebs of oil distributed over an area of about 4 sq cm. The females, on the other hand, still presented externally visible humps, though these were, to be sure, smaller than before.

Following this experience, an attempt has been made to gain a first approximation to the amount of oil that may be recovered under these circumstances, and to determine also the effect of castration. For this experiment stock laboratory Swiss mice were used. The gonads were removed from 10 males and 10 females at the age of 22-23 days; *i. e.,* before puberty. Two months later these animals, together with groups of normal young adult males and females of about the same age, were started on a series of injections of Mazola. The oil was given in doses of 0/1 ml into the subcutaneous tissues of the left flank, biweekly until a total of 1 ml had been administered to all of the mice. Seven or 8 days after the last injection the animals were killed. (Attention should be called at this point to the fact that while the same gross dissimilarity between males and females appeared in this strain of mice, it was not as striking as in the Bagg albinos. Evidently in the Swiss mice the amount of oil remaining unabsorbed is smaller in both sexes.) The blebs of oil, together with the adjacent overlying skin may be dissected away from the muscular sac containing the abdominal and thoracic viscera and spine without trouble. The oil was found to lie in thin capsules attached to subcutaneous tissue in area about the size of a quarter. This piece of skin was cut away, transferred to a Petri dish and extracted with anhydrous ether. The blebs were opened by scarification of the subcutaneous surface, and after a period of 5 minutes the ether was pipetted into a tube, centrifuged quickly at high speed to throw down hair and other solid particles, then finally transferred to a weighing bottle. Following evaporation and desiccation over $CaCl_2$ the quantity of material remaining was determined by weight. The results are presented in Tables I and II.

In the first place the rate of disposal of injected oil is fairly rapid in

TABLE I.
Ether-Soluble Material Recovered from Normal Young Adult Swiss Mice, mg.

		Female		Male
	++	126	+	110
	++	192	+	113
	++	209	++	116
	++	261	+	152
	++	311	+	176
	++	329	++	190
	++	350	++	224
Mean		254 ± 20.8		154 ± 11.3
$\sqrt{\frac{\Sigma d^2}{n-1}}$		81.7		44.2
Diff. of Means		100 ± 23.7		
x P.E.	4.2			

+ - ++ indicates gross estimate of the relative amount of oil.

TABLE II.
Ether-Soluble Material Recovered from Castrate Young Adult Swiss Mice, mg.

		Female		Male
	+	167	+	152
	++	179	++	179
	++	205	+	232
	+	217	++	233
	+	239	++	249
	++	270	+	276
	++	294	++	300
	++	322	++	304
	++	380	+	308
	+	487	++	383
Mean		276 ± 21.1		262 ± 14.4
$\sqrt{\frac{\Sigma d^2}{n-1}}$		99.1		67.6
Diff. of Means		14 ± 25.5		
x P.E.	.55			

+ - ++ indicates gross estimate of the relative amount of oil.

all the groups examined. For, even if it be assumed that all of the material recovered is vegetable oil, and if the specific gravity of the oil is about 0.9, then the maximum average recovery did not in any case exceed 30% of the quantity administered (900 mg). Actually, of course, some normal subcutaneous fat is extracted and contributes to the size of the figures given.

Secondly, there appears to be a significantly smaller average amount of ether-soluble material obtained from the normal males as compared to the normal females. Thirdly, after castration this sex differential is no longer apparent.

It is unnecessary to point out that the number of animals comprising this study is small and that there are errors inherent in the rather crude method of extraction. For instance, the figures given for the castrates are not to be strictly compared with the figures for the normal animals. The larger fat pad which develops after castration would, in any case, increase the quantity of ether-soluble materials obtained, regardless of the relative amounts of oil present. However, these data represent only the results of a preliminary effort to measure the rate of disposal of the oil, and do provide at least an approximation of the magnitudes involved.

In support of the idea that these observations reflect a relationship between sex hormones and intermediate metabolism of fat, mention may be made of so-called lipemia in birds. The subject has been recently reviewed[1] and need be examined here quite briefly. Estrogens exert a specific action on the blood fat of both male and female birds, producing enormous increases in the lipid content of the blood. Neutral fats, as well as phospholipids and cholesterol, share in the increase. Androgens do not have this effect. Riddle remarks, "Despite great gaps in our knowledge, particularly in the case of mammals, it is of much importance that estrogenic hormones have now been shown to have surprising ability to increase the blood fat in birds."[1] The method used in the experiments described above may prove of value for further exploration of the problem.

Summary. 1. Vegetable oil administered parenterally to mice is not physiologically inert but disappears rather rapidly from the site of injection. 2. The rate of disappearance of oil appears to vary to some extent in different breeds of mice. 3. The rate of disappearance of oil in both of two different strains of mice appears to be higher in normal young adult males than in normal females of the same age. 4. In a small series of mice, castration before puberty abolished this sex differential.

---

[1] Riddle, O., *Ann. Rev. Phys.*, 1941, **3**, 588.

13401

Acceleration of Hemolysis in Relation to Chemical Structure. III. The C-10 Alcohols.

ERIC PONDER.

From the Nassau Hospital, Mineola, L.I.

The investigation with which this note is concerned is a continuation of an inquiry into the relation between the chemical structure of various substances and the extent to which they accelerate hemolysis by simple hemolysins.[1] Through the kindness of Dr. F. C. Schmelkes I have been able to obtain purified samples of 4 C-10 alcohols, all of which, to a greater or lesser extent, are accelerators of saponin hemolysis.

Method. This has been described,* and consists in comparing time-dilution curves for the lysin (saponin) acting on washed red cells (oxalated rabbit blood) in the presence of varying quantities of the accelerator. The comparison gives a constant R; (R-1) divided by the concentration *c* of the accelerator in mM/l provides a satisfactory measure of the accelerating power of the substance.

The solubility of the C-10 alcohols is very small, and great care must be taken that they are really in solution. The procedure is to add a weighed quantity of the C-10 alcohol dissolved in 1 ml of ethyl alcohol to 1000 ml of water in a large glass-stoppered bottle. The bottle with its contents is vigorously shaken every hour or so for several days, first at room temperature and then when warmed to about 60°C, or until the material appears to be completely dissolved. If it is not completely dissolved, a smaller quantity is weighed out, and the process repeated. Sufficient NaCl is added finally to make the solution isotonic. The small amount of ethyl alcohol present has no accelerating or inhibiting effect on saponin hemolysis. The maximum concentration c_m obtained and used in the case of each alcohol, together with the molecular weights (M.W.) and the density (D), are given in Table I.

Saponin in dilutions of 1 in 10,000 to 1 in 60,000 in 1% NaCl was used as the lysin throughout, and the time-dilution curves were obtained at 25°C. In analyzing them, I encountered the difficulty of a diminishing R-value near the asymptotes; this complication has

---

[1] Ponder, E., *J. Exp. Biol.*, 1939, **16**, 38, acceleration by benzene derivatives; Ponder, E., and Hyman, C., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 320, acceleration by straight-chain alcohols.

* Published in *J. Exp. Biol.*, 1939, **16**, 38.

TABLE I.
Accelerating Power of C-10 Alcohols for Saponin Hemolysis.

	M.W.	D.	c_m mM/l	(R — 1)/c	× benzene (0.025 mM/l)
Nonyl alcohol	144	0.84	0.5	—0.8	32
n-decanol	158	0.83	0.2	—2.1	83
Tetrahydro-geraniol	158	0.86	0.2	—1.0	40
Citronellol	156	0.88	0.2	—0.6	24
Geraniol	154	0.85	0.2	—0.1	4

been fully discussed elsewhere,[2] and the R-values given in Table I are those obtained from parts of the curves well below the asymptotes, *i. e.*, where R is relatively constant. In the last column of the table, each accelerator is compared with benzene as regards potency, benzene having been used as a kind of reference substance in work of this kind.

Results. These are shown in Table I, and lead to 2 conclusions. 1. The accelerating power of the straight-chain C-10 alcohol, n-decanol, is considerably less than that calculated from the expression given by Ponder and Hyman as relating the accelerating power $A = (1\text{-}R)/c$, to the number of carbon atoms N. The expression, $N = 1.75 \,.\, \log A + 9$, gives 9.6 for N instead of 10, or, to have $N = 10$, A would have to be 3.7 instead of 2.1; the observed accelerating power is accordingly only about half of that predicted. 2. Among the C-10 alcohols themselves, small differences in structure correspond to considerable differences in accelerating power. Thus tetrahydro-geraniol, a branched chain alcohol with the same molecular weight as n-decanol, produces only about half the acceleration of the latter, whereas the introduction of one double bond, as in citronellol, reduces the accelerating power still further, and the presence of two double bonds, in geraniol, almost abolishes it. In most instances where comparisons of this kind have been made, the differences in accelerating power have corresponded to greater differences in molecular structure (the length of the carbon chain, the number of added halogens, etc.) than we have here.

Conclusion. The accelerating power of 4 C-10 alcohols on saponin hemolysis is in the order of n-decanol>tetrahydro-geraniol>citronellol>geraniol, these differences corresponding to small structural differences in the accelerating molecule.

---

[2] Ponder, E., *J. Gen. Physiol.*, 1941, **25**, 247.

13402

Effect of Dilution of Neutral Mixtures of Eastern Equine Encephalomyelitis Virus and Antiserum.*

Marjorie E. Pierce, J. Emerson Kempf and Malcolm H. Soule.

From the Hygienic Laboratory, University of Michigan, Ann Arbor.

Neutral mixtures of a number of viruses and their homologous antisera have been found to be reactivated by simple dilution with 0.85% saline solution.[1–10] The same phenomenon holds true for bacteriophage-antibacteriophage serum,[11, 12] toxin-antitoxin,[13–16] as well as a neutral mixture of the pneumococcus type III.[17]

The work of Merrill[18] suggested an exception to the above general findings. In his report, a mixture of eastern equine encephalomyelitis virus and serum composed of a 10% suspension of guinea pig brain and undiluted immune horse serum when serially diluted 10^{-1} to 10^{-5} did not produce infection when injected intraperitoneally into mice. The question as to whether or not this particular virus-serum mixture becomes active on dilution has an important bearing on neutralization tests which are so frequently used in research with this agent of disease and seemed worthy of reinvestigation.

---

* This work was aided by a grant from the Clara Ward Seabury Clinic for the Study of Infantile Paralysis.

[1] Downie, A. W., and McGaughey, C. A., *J. Path. and Bact.*, 1935, **40**, 297.

[2] Todd, C., *Brit. J. Exp. Path.*, 1928, **9**, 244.

[3] Andrewes, C. H., *J. Path. and Bact.*, 1928, **31**, 671; 1932, **35**, 242.

[4] Bedson, S. P., *Brit. J. Exp. Path.*, 1928, **9**, 235.

[5] Burnet, F. M., *J. Exp. Med.*, 1936, **63**, 685.

[6] Burnet, F. M., Keogh, E. V., and Lush, D., *Australian J. Exp. Biol.*, 1937, **15**, 320.

[7] Taylor, R. M., *J. Immunol.*, 1941, **40**, 373.

[8] Schultz, E. W., Gebhardt, L. P., and Bullock, L. T., *J. Immunol.*, 1931, **21**, 171.

[9] Findlay, G. M., *Brit. J. Exp. Path.*, 1936, **17**, 89.

[10] Strong, P. S., *J. Immunol.*, 1936, **30**, 403.

[11] Otto, R., and Munter, H., *Z. f. Hyg.*, 1922, **98**, 302.

[12] Andrewes, C. H., and Elford, W. J., *Brit. J. Exp. Path.*, 1933, **14**, 376.

[13] v. Behring, E., *Beitr. Z. Exp. Therap.*, 1904, H.**7**, 51.

[14] Madsen, T., *Z. f. Bakt.*, 1 Ref., 1906, **37**, 373.

[15] Otto, R., and Sachs, H., *Z. f. Exp. Path. u. Therap.*, 1906, **3**, 19.

[16] Glenny, A. T., *J. Hyg.*, 1925, **24**, 301; Glenny, A. T., Pope, C. G., and Waddington, H., *J. Path. and Bact.*, 1925, **28**, 279; Glenny, A. T., and Barr, M., *J. Path. and Bact.*, 1932, **35**, 142.

[17] Enders, J. F., and Shaffer, M. F., *J. Immunol.*, 1937, **32**, 379.

[18] Merrill, M. H., *J. Immunol.*, 1936, **30**, 185.

In carrying out the studies, advantage was taken of the various contributions of Olitsky and colleagues[19–21] which emphasized the importance of the intraperitoneal route of inoculation in mice. These workers showed this avenue to be the most effective method for demonstrating the protective power of the serum but called attention to the necessity of using 15-day-old albino mice which are highly susceptible to the virus.

Methods. The virus used was a strain of eastern equine encephalomyelitis obtained from Dr. Jerome T. Syverton in 1938. It has been maintained by intracerebral passage in mice with frequent storage of the infected brain tissue in 0.85% saline solution at 10°C. Before each experiment the virus was given several passages in mice to insure a high virulence, and the mld of each preparation was determined.

Immune serum was obtained from a rabbit which had received an intravenous injection of a 10% suspension of virus, 2 injections of Lederle vaccine by the same route and one intraocular injection of virus. Several rabbits had been immunized in this manner for other studies and the one showing the highest antiviral titer was selected for the present work. The rabbit was bled at intervals by cardiac puncture, and the serum without preservative was stored in the refrigerator (10°C).

To prepare the virus for the virus-serum mixture, a 20% suspension of infected brain tissue made by grinding with sand and diluting with 0.85% saline solution was centrifugated at 1600 rpm for 20 minutes in the angle head centrifuge. The supernatant solution was diluted with 0.85% saline solution to the desired concentration and added in equal amounts to undiluted immune serum and after thorough shaking was allowed to stand at 26°C (room temperature) for 15 minutes. The neutrality of each mixture was determined by mouse injection. To determine the influence of dilution on reactivation, the desired amount of 0.85% saline solution was added and incubation carried out at room temperature for 15 minutes. Mice were then injected, each mouse receiving 0.1 cc of each dilution intraperitoneally. The animals used were either *P. maniculatus* under 90 days of age, which have been found in this laboratory to be highly susceptible to the virus when introduced by the intraperitoneal route, or 15-20-day-old albinos. Two controls

---

[19] Cox, H. R., and Olitsky, P. K., *J. Exp. Med.*, 1936, **64**, 217.

[20] Olitsky, P. K., and Harford, C. G., *J. Exp. Med.*, 1938, **68**, 173.

[21] Sabin, A. B., and Olitsky, P. K., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 597.

were employed: (1) the determination of the potency of the virus when mixed with an equal volume of undiluted normal rabbit serum, and (2) tests for the neutralization of the virus by the immune serum as mentioned above. The titer of the virus varied between 10^{-6} to 10^{-7} cc. Over-neutralized, neutral, and under-neutralized mixtures were obtained by the use of concentrations of virus as follows: 1:1,000, 1:100, and 1:10 in the presence of an equal volume of undiluted immune serum. The data for 7 experiments have been combined and are presented in Table I.

Discussion. It is strikingly evident from the data in Table I that the virus in the neutral mixture was reactivated following dilution of 1:100 with 0.85% saline solution. Nineteen of the 27 mice injected with the diluted material died with typical signs of eastern equine encephalomyelitis. In contrast only 3 of the 26 animals injected with the undiluted material succumbed. Confirmation of the cause of death of the animals injected with the diluted material was obtained in two ways: (1) by mouse passage of bacteria-free brain tissue with the production of characteristic signs of this infection, (2) protection tests against this passage virus with the specific antiserum.

Now, turning to the mixtures in which the dilution of the virus was 1:1,000, further dilution evoked no detectable reactivation. If one calculates the titer of virus in the final dilution, the quantity injected should still be adequate to kill the mice if dissociation of virus and antivirus were complete. The controls on this dilution show an ex-

TABLE I.
Reactivation of Mixtures of Eastern Equine Encephalomyelitis Virus and Antiserum by Dilution with Saline Solution.

Conc. of virus in original virus-serum mixture at time of dilution	Dilution of virus-serum mixture tested for reactivation	Results of test for reactivation
1:1000	1:10	1/21*
”	1:100	3/27
”	1:200	0/5
”	undiluted (control)	1/15
1:100	1:10	6/18
”	1:100	19/27
”	1:1000	0/3
”	undiluted ”	3/26
1:10	1:10	9/12
”	1:100	11/12
”	undiluted ”	8/13

*The denominator indicates the total number of animals injected and the numerator the number of deaths.

cess of antiserum. Under these conditions, as might be expected, reactivation rarely occurs. In the mixture containing 1:10 virus we have the other extreme, namely, virus in excess. The injection of this material produced death even when diluted, merely confirming the potency of the virus.

The importance of having balanced mixtures of virus and antiserum in order to demonstrate reactivation by dilution is clearly brought out in the table. If antiserum is in excess, there is no detectable liberation of virus, and when virus is in excess the mixture is naturally potent. These findings agree with the work of Goyal[22] on vaccinia virus and possibly account for the negative results of Merrill. The extent of dilution is also a factor since the mixture containing virus in a concentration of 1:100 was not reactivated when further diluted 1:10 and 1:1000 but was reactivated in a dilution of 1:100.

Conclusions. 1. A neutral mixture of eastern equine encephalomyelitis virus and homologous antiserum was reactivated when a 1:100 concentration of virus with antiserum was diluted a hundredfold with 0.85% saline solution and was allowed to stand at 26°C (room temperature). 2. It appears that reactivation will occur only in a balanced mixture of virus and serum and furthermore, the extent of dilution is important.

13403

Effects of Repeated Small Doses of Roentgen Rays on Canine Blood and Bone Marrow.

R. M. Mulligan. (Introduced by H. J. Clausen.)

From the Department of Pathology, School of Medicine and Hospitals, University of Colorado, Denver.

Since the human and experimental data on the effect of roentgen rays on the blood and the bone marrow deal with large doses and their sequelae over relatively short periods of time, 4 dogs were exposed to doses of 75 r or 100 r units administered at intervals of a week or longer over several months.

Before irradiation, dogs 11, 21 and 24 were given a course of subcutaneous benzol injections (preceded by 4% novocaine local anesthesia) over 12 weeks. This project was abandoned because of diffuse subcutaneous edema in dog 11 between the 8th and 12th weeks and in dog 24 between the 3rd and 8th weeks, and the development of draining sinuses in dog 21 between the 2nd and 8th weeks which had nearly completely healed at the 12th week.

---

[22] Goyal, G. K., *J. Immunol.*, 1935, **29**, 111.

The data for the benzol experiments are illustrated to contrast with those following irradiation which was given under nembutal anesthesia over the left side to overspread the caudal half of the left thorax and the cranial half of the left abdomen with the animal resting on its right side. A portal of 15 x 15 cm, a focal skin distance of 50 cm, 215 K.V., 15 m.a., filtration of ½ mm of Cu and 1 mm of Al, and 2½ min for 100 r were used. Venous blood samples were oxalated before each exposure for the counts recorded. The bone marrow was obtained by rib resection.[1]

In all animals the first line on the y-axis of the graph and row I in the table show the values noted before any experimentation was begun. In the graph, the arrows pointing up show individual rib resections which correspond to the bone marrow values presented in the table in chronological and Roman numerical order. The vertical lines indicate doses of benzol, 2 cc each for dogs 11 and 24 and 3 each for dog 21. The longer arrows pointing down mark doses of 100 r units of X-ray; the shorter arrows pointing down, 75 r units. The figures along the x-axis indicate weeks for benzol injections and days for X-ray exposures. In the table, N means normal; mark hype, markedly hyperplastic; mod hypo, moderately hypoplastic; C.I., cellularity indeterminable; hype, hyperplastic; and hypo, hypoplastic.

In dog 11 the total leucocytes in thousands per cu mm (WBC) were depressed, except for an occasional rise, at a level of 5,000 or slightly below. After 400 r units had been received, a marked rise in the percentage of stab neutrophiles (StN) and a proportionate decrease in the segmented neutrophiles were seen. The lymphocytes ranged generally from 12-26%, but occasionally dipped as low as 6%. The eosinophiles reached a few sharp peaks, the highest 17%. The monocytes, not plotted, ranged between 0-5%. During irradiation, metamyelocyte neutrophiles were intermittently present between 1-4%. Basophiles, never found in normal dogs,[2] occupied 1-2% of some counts. In these experiments they are believed to be neutrophiles inclosing basophilic degeneration granulations. Sporadically, a few of both the polymorpho- and mononuclear forms

---

[1] Mulligan, R. M., *Anat. Rec.*, 1941, **79**, 101.

[2] Mulligan, R. M., *Am. J. Physiol.*, 1941, **133**, 394.

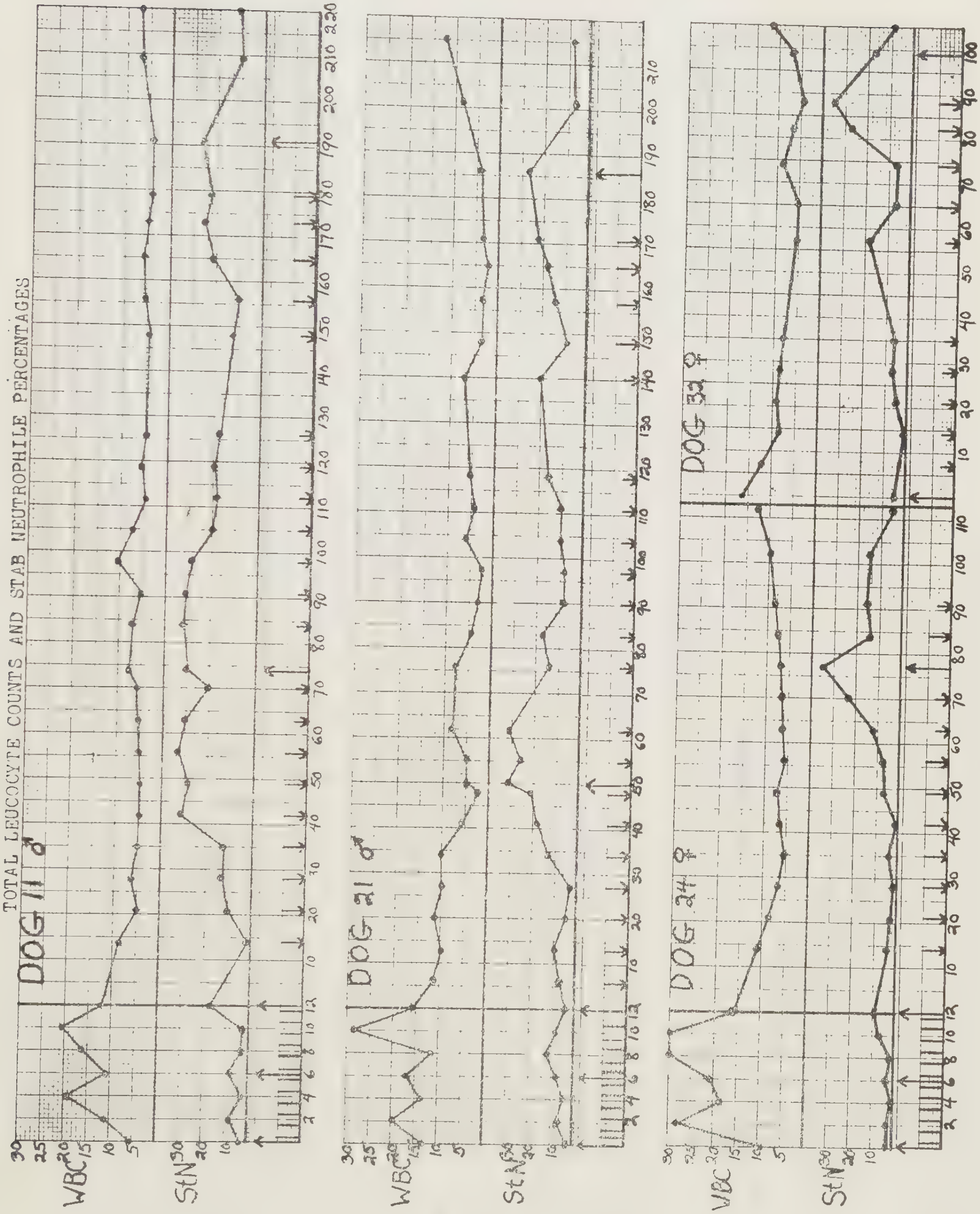


FIG. 1.

had bizarre nuclei and vacuolated cytoplasm. These and other damaged forms were not included in the counts. The erythrocytes were depressed from 5.5 to 4.5 million per cu mm after 850 r units. Corresponding changes in the hemoglobin (Newcomer) in grams

TABLE I.
Summary of Bone Marrow Studies.

	Eosinophiles	Promyelocyte neutrophiles	Myelocyte neutrophiles	Metamyelocyte neutrophiles	Stab neutrophiles	Segmented neutrophiles	Proerythro-blasts	Erythroblasts	Normoblasts	Stem cells	Lymphocytes	Unidentified cells	Myeloid / Erythroid ratio	Cellularity
Dog No. 11														
I	4.6	2.0	4.6	5.6	24.6	4.0	0.8	1.6	46.4	1.4	0.8	3.6	0.9	N
II	3.0	2.2	4.0	13.0	33.4	2.2	0.2	0.6	37.0	0.6	1.6	1.8	1.5	N
III	2.0	1.8	3.8	17.4	26.4	1.8	0.8	1.4	42.0	0.4	0.8	1.4	1.2	mark hype
IV	2.0	0.8	2.0	6.6	11.0	0.6	0.6	1.8	70.0	0.2	2.2	2.2	0.3	mod hypo
V	2.4	0.4	3.0	7.6	16.0	1.0	0.2	2.6	61.2	0.0	3.6	2.0	0.5	mod hypo
Dog No. 21														
I	5.2	1.2	3.4	7.8	25.4	3.0	0.4	3.0	48.2	0.6	1.0	0.8	0.9	N
II	5.4	3.4	11.8	15.2	41.4	7.2	0.0	1.2	8.4	1.6	1.8	2.4	9.0	C.I.
III	2.0	1.4	7.0	18.2	35.4	4.4	0.0	0.8	26.2	0.2	2.0	2.4	2.5	mod hype
IV	2.4	0.2	1.8	9.4	23.0	1.0	0.0	2.2	56.4	0.0	2.0	1.6	0.6	hypo
V	2.6	0.0	4.0	11.4	22.0	1.2	0.4	3.4	52.2	0.0	1.2	1.6	0.7	hypo
Dog No. 24														
I	4.0	1.2	4.2	7.2	26.0	4.4	1.0	2.6	45.6	0.8	1.4	1.6	1.0	C.I.
II	4.4	2.4	7.8	14.2	30.8	3.2	0.0	0.2	29.2	0.8	3.8	3.2	2.1	hype
III	3.0	2.4	7.0	9.2	13.4	2.2	0.4	2.0	53.2	0.4	2.0	4.6	0.7	N
IV	3.0	1.4	3.6	9.6	18.2	2.4	0.8	1.8	53.8	0.6	2.8	2.0	0.7	mod hypo
Dog No. 32														
I	4.4	1.0	4.2	13.2	27.8	3.6	0.6	2.2	37.8	0.4	2.2	2.4	1.3	N
II	4.2	0.8	2.2	9.0	17.8	1.4	0.8	2.2	58.2	0.2	2.0	1.2	0.6	mod hypo

per 100 cc of whole blood were noted. Several times a high mean corpuscular hemoglobin was associated with hyperchromia of the erythrocytes. Normoblasts were irregularly noted up to 2%. The bone marrow after irradiation exhibited moderate hypoplasia and definite lowering of the myeloid/erythroid ratio.

At the same dosages (400 r and 850 r) dog 21 behaved essentially as dog 11, exhibiting leukopenia as low as 2,200, a lower average level of lymphocytes, 8-16%, and a drop in erythrocytes from 6.2 to 4.8 million per cu mm. The bone marrow of this animal after 700 r units was obviously hypoplastic. The myeloid/erythroid ratio of 9.0 after 6 weeks of benzol injections was undoubtedly due to the presence of the infected draining sinuses mentioned.

Dog 24 showed similar findings except for the absence of eosinophilia and of metamyelocyte neutrophiles in the blood. The lymphocytes ranged between 12-30%. The erythrocytes dropped from 6.4 to 4.6 million per cu mm after 950 r. After having received 1,025 r units, this animal was in heat, was mated with dog 11 on the 112th day of the experiment for each animal and delivered a normal litter of 2 male and one female puppies 58 days later. These nursed vigorously and are healthy at 4 months of age. The amount and timing of the X-ray exposure was not deleterious to the normal ovarian activity of this animal, since the ovaries lay directly in the path of the rays.

Dogs 11, 21, and 24 were irradiated on the same days until dog 24 was mated. Dog 32 was then substituted, received X-ray exposure on the same days as dogs 11 and 21, and demonstrated changes comparable to those seen in the other 3 animals. During the irradiation experiments, none of the animals evinced any disturbance whatsoever in appetite or weight; in the gastrointestinal tract; in the healing of wounds after rib resection; or in general activity. They were always fully recovered the day after anesthesia and X-ray exposure.

Summary. Repeated small doses of roentgen rays caused depression of the total leucocyte count, shift to the left in the neutrophile granulocyte line, occasional eosinophilia, and moderate anemia in the peripheral blood; hypoplasia and decrease in the myeloid-erythroid ratio in the bone marrow; and no evidence of interference with the general activity of the experimental animals.

Drs. J. W. McMullen, R. R. Anderson, and E. J. Meister of the Department of Radiology kindly administered the irradiation in these experiments.

13404 P

Brain Reactions to Bacterial Filtrates Potent in Eliciting Local Tissue Reactivity.*

Otto Marburg and Gregory Shwartzman.

From the Laboratories of the Mount Sinai Hospital, New York, and the Laboratory for Research in Neuropathology, Montefiore Hospital, New York

The effects of bacterial toxins upon neuraxis have received the attention of many investigators. Studies of Claude[1] (tetanus toxin); Putnam, McKenna and Evans[2] (tetanus toxin); Lotmar[3] *(Shigella dysenteriae),* and Branham and Lillie[4] (experimental meningitis) are of direct interest.

In the work described below bacterial principles potent in the elicitation of the phenomenon of local tissue reactivity were used. All injections were given only intravenously, thus avoiding the possible mechanical injury to the brain. It was shown previously that, while in its usual form the phenomenon requires for elicitation the combined local preparatory and intravenous provocative injections, in some organs 2 intravenous injections 24 hours apart are capable of producing severe lesions (kidneys, intestines, etc.) (Apitz,[5] Gerber,[6] and Shwartzman.[7]) In the latter modification the first intravenous injection takes the place of the local preparatory injection of the former. The report deals with histological examinations of the brain of 11 rabbits. Each intravenous injection was given in a dose of 1 cc per kilo of body weight of bacterial filtrates variously diluted. One rabbit received one intravenous injection of meningococcus "agar washings" filtrate diluted 1:5 and died 24 hours later. In this animal there was found an acute meningococcus encephalitis. In addition to severe perivascular infiltration there were some independent foci beyond the vascular site in the tissues. They predominated in the cerebrum, were less conspicuous in the brain stem, were

---

* Aided in part by a grant from the Rockefeller Foundation.

[1] Claude, H., *Arch. phys. norm. and pathol.*, Paris, 1897, **29**, 843.

[2] Putnam, T. J., McKenna, I. B., and Evans, J., *J. Psychol. and Neurol.*, 1932, **44**, 460.

[3] Lotmar, F., *Nissl-Alzheimer Arb.*, 1918, **6**, 248.

[4] Branham, S. E., and Lillie, R. D., *Pub. Health Rep.*, 1932, **47**, 1683.

[5] Apitz, K., *J. Immunol.*, 1935, **29**, 255.

[6] Gerber, I. E., *Arch. Path.*, 1936, **21**, 776.

[7] Shwartzman, G., *Phenomenon of Local Tissue Reactivity and Its Immunological, Pathological and Clinical Significance,* Medical Book Department of Harper & Brothers, 1937, New York.

absent from the cerebellum and contained leuco-, lymphocytes and many tissue cells showing marked disintegration of the nuclei (Fig. 1). Myelin-stained sections of the frontal brain disclosed small foci of demyelinization around the blood vessels (Fig. 2). The axons were swollen or destroyed. Another animal received 2 intravenous injections of meningococcus filtrate each in dilution 1:40 and died 24 hours after the second injection. There were foci of glia cells, histiocytes, leuco- and lymphocytes without any noticeable reaction around the blood vessels next to the ventricles but not in the fusion line. Two rabbits receiving single intravenous injections of meningococcus filtrate diluted 1:5 and 1:40 respectively, and 2 rabbits receiving each 2 injections of meningococcus filtrates diluted 1:5 and 1:40 respectively, showed an inflammatory irritation of the nervous system with pronounced changes in the glia cells and swelling of

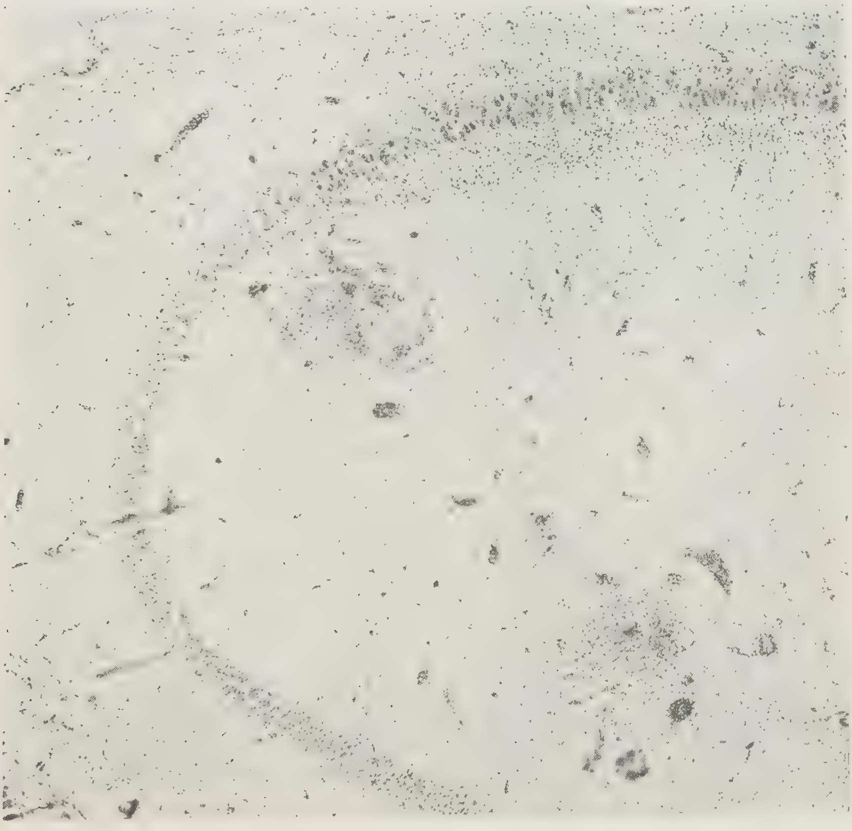


FIG. 1.
Rabbit 1: Foci in the hippocampal region. (Nissl stain.)

ganglion cells. Two rabbits receiving single intravenous injections of *Eberthella typhosus* "agar washings" showed only an increased number of damaged glia cells, mostly around the Purkinje cells and the molecular layer of the cerebellum. Two rabbits were injected with meningococcus toxin diluted 1:40 and reinjected 24 hours later with *Eberthella typhosus* toxin in dilution 1:10. They were killed 24 hours after the second injection. No lesions were found in the brain.

Thus, strong reactions scattered throughout the tissue itself of the entire brain and around the ventricles were similar to changes in the skin and kidneys of the phenomenon of local tissue reactivity. It is also noteworthy that the meningococcus toxin involved the white and gray matter alike.† Previous investigations showed that the phenomenon of local tissue reactivity deals with bacterial toxins capable of producing primary lesions in susceptible tissues. In tissues with natural resistance the preparatory effect of the first injection consists in the elicitation of reactivity permitting the production

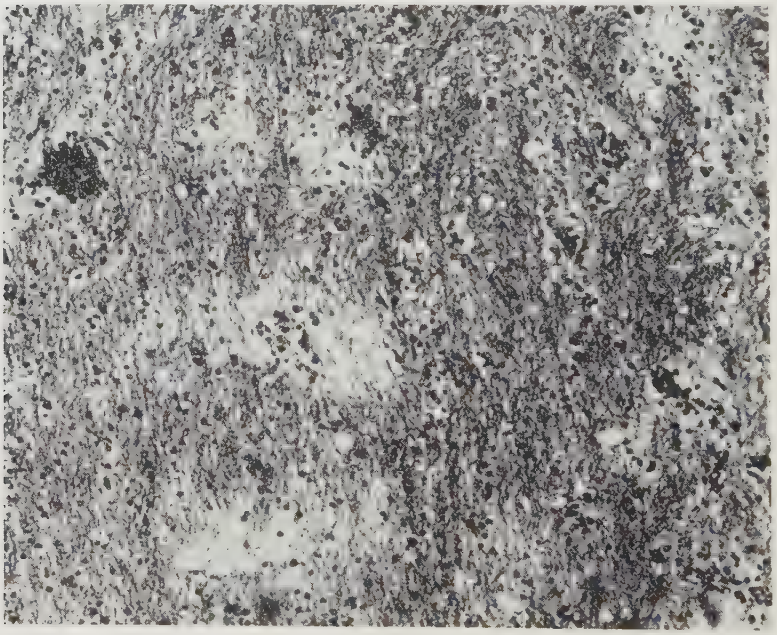


FIG. 2.
Rabbit 1: Frontal brain, demyelination. (Heidenhain stain.)

---

† According to Lotmar[3] (Fig. 3) *Shigella dysenteriæ* toxin damages the gray matter. The tetanus toxin, according to Claude[1] and to Putnam, McKenna and Evans,[2] affects rather the white matter.

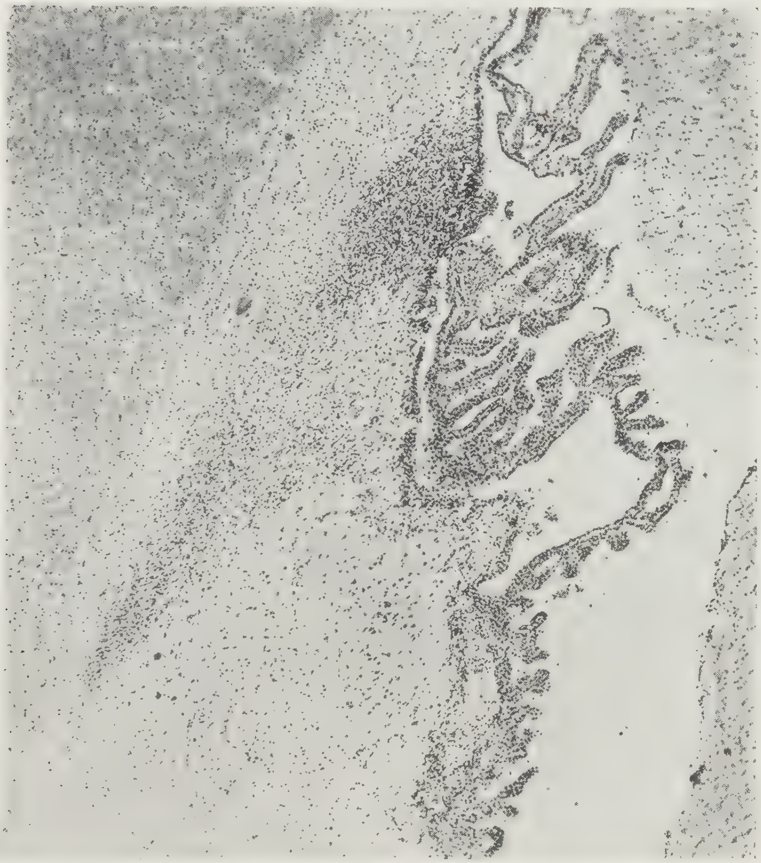


Fig. 3.
Rabbit 2: Foci around the ventricle. (Haemalum and eosin stain.)

in the prepared sites of severe lesions by means of the following provocative injections. In this manner "normal" endogenous bacterial inhabitants may evoke serious damage in refractory tissues and organs. A single intravenous injection and invasion of the blood stream by bacteria may induce damage in tissues and organs made previously reactive by the presence of spontaneous infections.[7]

The only conclusion now possible is that the bacterial filtrates potent in the elicitation of the phenomenon of local tissue reactivity may also produce severe damage in the brain of rabbits by way of the blood stream. The possible enhancement and modification of the character of lesions seen in the phenomenon in other organs have not been ascertained as yet in studies on the brain.

13405

Reduction of 2-(p-Nitrobenzenesulfonamido)-Pyridine to Sulfapyridine in the Animal Body.

C. J. Weber, J. J. Lalich* and R. H. Major.

From the Hixon Laboratory for Medical Research, University of Kansas, School of Medicine, Kansas City, Kansas.

The substitution of a nitro for the amino group in sulfonamides, produces compounds with bactericidal activity *in vivo*[1, 2] and *in vitro*.[3] Flynn and Kohl[4] have shown that p-nitrobenzenesulfonamide is capable of reduction in the body. We became interested in 2-(p-nitrobenzenesulfonamido)-pyridine[†] because of its apparent lack of toxicity on oral administration in spite of its antistreptococcal activity *in vivo*.[5] This finding was of sufficient interest to warrant a study of the fate of 2-(p-nitrobenzenesulfonamido)-pyridine after oral and intravenous administration in the dog, and following oral administration in man, rabbit, and the rat.

2-(p-Nitrobenzenesulfonamido)-pyridine is an insoluble, almost colorless compound which melts with decomposition at 192-3°C (corrected). On reduction with tin and HCl, sulfapyridine is obtained. The solubility in water is less than 1 mg % at 20°C.[‡] This compound has also been described by Burton, *et al.*, but they did not give any specific data except to state that due to its insolubility *in vitro* studies were difficult.

In order to study the fate of 2-(p-nitrobenzenesulfonamido)-pyridine in the animal body, the following procedure was used in determining this compound in the blood. Two cc of blood were diluted to 40 cc with distilled water and placed in a boiling water bath for 3 minutes. Seven drops of 1% acetic acid were added, and the tube was heated in the bath for another 3 minutes. After cooling, it

---

[*] George A. Breon Fellow in Experimental Medicine.

[1] Mayer, R. L., and Oechslin, C., *Compt. Rend. Acad. Sc.*, 1937, **205**, 181.

[2] Buttle, G. H., Foster, T. D. G. E., Gray, W. H., Smith, S., and Stephenson, D., *Biochem. J.*, 1938, **32**, 1101.

[3] Burton, H., McLeod, J. W., McLeod, T. S., and Mayr-Harting, A., *Brit. J. Exp. Path.*, 1940, **5**, 288.

[4] Flynn, L. M., and Kohl, M. F. F., Proc. Soc. Exp. Biol. and Med., 1941, **47**, 466.

[†] These nitro compounds have been kindly supplied by George A. Breon and Co.

[5] Unpublished data.

[‡] Personal communication with Doctor H. P. Brown (1940).

was diluted to 50 cc, mixed, and filtered. Bratton and Marshall's method[6] was applied, using 9 cc filtrate and 1 cc 4 N HCl. Another portion of filtrate (18 cc plus 2 cc 4 N HCl) was shaken for 5 minutes with 0.5 g zinc dust to effect a reduction, filtered, and 10 cc of this filtrate was used for the color determination. The difference between these two values gave a very good index of the quantity of 2-(p-nitrobenzenesulfonamido)-pyridine present. Determinations in the urine were made by employing appropriate dilutions of urine and treating the diluted urine in the same manner. After adding 2-(p-nitrobenzenesulfonamido)-pyridine to blood, it was possible by this method to account for 85-90% of the compound present.

The toxicity of orally ingested 2-(p-nitrobenzenesulfonamido)-pyridine in the dog, rat, or mouse is practically *nil*. This low toxicity is apparently due to poor absorption as shown by the low blood level. After administering 0.5 g/kg suspended in 5% acacia in a single dose, the highest blood level reached in 3 hours was 1.1 mg %. Following repeated doses of 0.2 g/kg at 2-hour intervals for 10 hours, we succeeded in raising the blood level to 4.0 mg %. Slow intravenous injection of a 0.1% solution of 2-(p-nitrobenzenesulfonamido)-pyridine in alkaline saline (.005 N NaOH) produced toxic reactions manifested in the form of vomiting and salivation, when the blood level reached 6-7 mg %. At a level of 10-11 mg % defecation and violent retching became evident. These symptoms subsided in the course of 3-4 hours without any apparent harm to the animal.

A striking difference was shown in toxicity of p-nitrobenzenesulfonamide and 2-(p-nitrobenzenesulfonamido)-pyridine following oral administration. This difference is due to two factors: more rapid absorption of p-nitrobenzenesulfonamide, and an apparent inability of the kidney to eliminate this compound. The blood levels at which toxicity became apparent were essentially the same for both drugs. Following oral doses of 0.1 g/kg of p-nitrobenzenesulfonamide the dogs manifested toxic symptoms and showed high blood levels over a period of 24-48 hours due to the slow excretion from the kidney. Even though 2-(p-nitrobenzenesulfonamido)-pyridine was poorly absorbed we found that Marshall's method showed a considerable amount of the reduced compound in the blood. The concentration of the reduced compound did not reach its highest peak until 12 hours after the oral administration of 2-(p-nitrobenzenesulfonamido)-pyridine. Following intravenous administration, although the level of 2-(p-nitrobenzenesulfonamido)-pyridine was

---

[6] Bratton, A. C., and Marshall, E. K., Jr., *J. Biol. Chem.*, 1939, **128**, 537.

TABLE I.
Comparison of Blood and Urine Levels in the Dog Following Administration of p-Nitrobenzenesulfonamide and 2-(p-Nitrobenzenesulfonamido)-pyridine.

Time in hr	2	4	8	12	24	48	72
p-Nitrobenzenesulfonamide							
Intravenous							
.06 g/kg 4-hr period							
Blood mg%							
N	4.0	5.8	4.7	4.1	2.8	1.4	0.6
R	0.2	0.4	1.0	1.2	1.4	1.1	0.4
Urine mg total excreted.							
N	10.2	27.4		45	54	66	74
R	2.0	8.5		74	162	287	375
Oral 0.1 g/kg in 2 doses							
Blood.							
N		5.5*	6.0*		4.0	2.0	1.0
R		1.0	1.0		1.5	1.3	1.0
Urine.							
N		20	22		22	22	22
R		4.0	8.0		186	372	489
2-(p-Nitrobenzenesulfonamido)-pyridine							
Intravenous							
0.1 g/kg 4 hr period							
Blood.							
N	1.7	3.3	0.8	0.5	0.4	0.4	
R	0.15	0.15	0.5	0.4	0.3	0.0	
Urine.							
N	152	297			798	815	
R	3.7	9.9			370	483	
Oral 0.5 g/kg single dose							
Blood.							
N		0.5	0.5	0.3	0.4		
R		1.0	2.0	3.2	2.8		
Urine.							
N			27		27		
R			114		514		

N—Nitro compound, R—Reduced compound. *Retching and Vomiting.

much higher than after oral administration the amount of reduced compound was much lower, suggesting that reduction occurs before the drug reaches the blood. Urinary excretion also reflected the greater reduction after oral administration.

We believe this difference can best be explained by assuming that much of the reduction of 2-(p-nitrobenzenesulfonamido)-pyridine takes place in the large intestine. To substantiate this assumption, incubation of dog's ileal and colonic contents mixed with 2-(p-nitrobenzenesulfonamido)-pyridine gave high values for reduced compound, while the contents of the stomach and duodenum showed only traces. From tryptose phosphate broth containing 2-(p-nitrobenzenesulfonamido)-pyridine inoculated with *B. coli communior* and incubated at 37.5°C for 5 days, we were able to isolate a compound having a melting point 190-191°C and a diazo-color value of sulfapyridine, hence one reduction product of 2-(p-nitrobenzenesulfonamido)-pyridine is sulfapyridine. Undoubtedly the greater part of the reduction takes place in the intestinal tract. However, macerated liver or kidney incubated at 37.5°C in a phosphate buffered solution (pH 7.4) also caused a reduction of 2-(p-nitrobenzenesulfonamido)-pyridine.

2-(p-nitrobenzenesulfonamido)-pyridine fed to rabbits was isolated from urine in the form of acetylsulfapyridine. Following oral administration of 7-8 g of 2-(p-nitrobenzenesulfonamido)-pyridine daily to man, free sulfapyridine was isolated from the urine.

We wish to point out that 2-(p-nitrobenzenesulfonamido)-pyridine may have a therapeutic application in the treatment of lower intestinal infection, since the reduction of 2-(p-nitrobenzenesulfonamido)-pyridine in the intestinal tract by *B. coli* would undoubtedly give rise to significant concentrations of sulfapyridine and probably to other reduction compounds which might be therapeutically effective.

13406

Injection of Heavy Water into the Cerebro-Spinal Fluid Space.*

KARL STERN AND T. E. DANCEY. (Introduced by D. McEachern.)
From the Verdun Protestant Hospital, Montreal, Que.

After Barbour[1] and his associates had demonstrated the sympathicomimetic action of heavy water in mice when 1/5 of the body water was replaced by heavy water it seemed interesting to study the effect of partial replacement of cerebrospinal fluid by normal saline made up of heavy water. The experiments were carried out in 2 akinetic catatonic patients (male, age 35, female, age 30). A certain amount (see below) of cerebrospinal fluid was withdrawn and replaced by a physiological saline solution made up in 99.5% deuterium oxide instead of water. This was done by lumbar puncture in the recumbent position and the head lowered during the injection. In the first case 2 cc and 3.8 cc of heavy water saline were thus injected at an interval of 6 days. In the second case 10 cc were injected first, 22 cc and 14.2 cc later with an interval of 14 days between each injection. The cerebrospinal fluid was examined 8 hours after

---

* This work was aided by a grant from the Medical Faculty of McGill University.

[1] Barbour, H. G., and Herrmann, J. B., *J. Pharm. and Exp. Ther.*, 1938, **62**, 158.

the first injection and thereafter at increasing intervals of 2 days to 3 weeks until 2 months after the last injection.

The results can be briefly summarized as follows: There was no appreciable change in the psychiatric or neurological picture, nor in the pulse rate, temperature, respiration, blood pressure, basal metabolic rate, blood cholesterol, sugar tolerance curve and kidney function. There was a marked cellular reaction in the cerebrospinal fluid. This was highest 8 hours after the injection (380 cells after 2 cc in case 1; 470 cells after 10 cc in case 2). This reaction changed within 2 days from a polynuclear one to a lymphocytic one. The cellular reaction was much stronger than could be expected from repeated lumbar puncture and it could be observed as long as 2 months after the last injection (7 cells per cmm). It was also more marked and prolonged than might be expected from equal amounts of normal saline. There was also, during the first 2 days, an increase in total protein (from 0.015 to 0.040 g %).

The long-standing cellular reaction is noteworthy because Herrmann[2] has shown that heavy water disappears from the cerebrospinal fluid spaces within 30 minutes, whereas meningeal reactions of this duration are commonly associated with the presence of slowly reabsorbed material in the cerebrospinal fluid spaces (Finlayson and Penfield[3]).

13407

Precipitative Tests in Malaria.*

ANNA DEAN DULANEY AND VIRGINIA HOUSE. (Introduced by L. A. Crandall.)

From the Departments of Bacteriology and Preventive Medicine, University of Tennessee College of Medicine, Memphis, Tennessee.

We have obtained precipitative reactions in human malaria with *P. knowlesi* antigens through use of the collodion-agglutination method of Cannon and Marshall,[1, 2] and the collodion fixation procedure of Goodner.[3]

---

[2] Herrmann, J B., *J. Pharm. and Exp. Ther.*, 1939, **67**, 265.

[3] Finlayson, A. I., and Penfield, W., *Arch. Neur. Psych.*, 1941, **46**, 250.

* This work was made possible through a coöperative program financed by the Tennessee Valley Authority and the University of Tennessee.

[1] Cannon, P. R., and Marshall, C. E., *J. Immunol.*, 1940, **38**, 365.

[2] *Ibid.*, 1941, **40**, 127.

[3] Goodner, Kenneth, *Science*, 1941, **94**, 241.

Cannon and Marshall[1, 2] adsorbed purified antigens on collodion particles. The sensitized particles were then combined with serial dilutions of antisera and the precipitative titers thus determined. By this method precipitins have been demonstrated in sera from patients sensitive to egg protein, tuberculin and crystalline insulin. In studies of the mechanism of the Arthus phenomenon precipitative titers were found to parallel skin reactivity. Weir[4] has also used this collodion agglutination procedure to show antibody formation in animals to tubercle bacilli and tuberculin.

Goodner[2] adds collodion particles at the time of combining antigen and antibody. The adsorption of the collodion pellets by the antigen-antibody complex produces particulate agglutination which may be macroscopically estimated. Antiviral and antimalaria antibodies have been demonstrated by this method.

Our previous attempts to demonstrate a precipitative reaction by overlaying sera from malarious patients with antigens prepared from parasitized bloods of human individuals and monkeys were unsuccessful. For these tests we used saline and watery extracts of malaria parasites and blood clots, as well as papain digests and sodium hydroxide extracts of parasites. We could not show significant differences between malarious and non-malarious sera.

A saline extract of *P. knowlesi* parasites obtained from heavily infected monkeys has proved a very satisfactory antigen for use in complement fixation tests in human malaria. Eighty-two percent of 125 patients with malaria parasites in the peripheral blood gave positive reactions although approximately one-third of this group had received some anti-malaria drug. This antigen as well as the alkaline extract of parasites described below has proved successful in the two procedures employing collodion particles.

The Use of Cannon and Marshall's Technic. In the collodion agglutination tests we followed exactly the technic of Cannon and Marshall[†] in all essential details. The antigen used for the tests reported in Table I was prepared by extracting 0.1 g of dried *P. knowlesi* parasites with 10 cc of N/20-N/10 NaOH for an hour. After neutralization it was centrifuged at high speed and the supernatant used to coat the collodion particles. It is recognized that this is a crude preparation but attempts to purify it by dialysis, or treatment with precipitating reagents have been unsuccessful. Difficulties with adsorption of antigen by particles which we encountered at times are attributed to the unpredictable variation of different lots of antigen.

---

[4] Weir, J. M., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 47.

[†] Dr. Cannon kindly supplied us with crystalline egg albumen and antiserum for our initial work.

The sera were used in dilutions beginning with 1:4. A control series employing the same serum dilutions and uncoated collodion particles was run simultaneously whenever the quantity of serum permitted. Table I presents the results of precipitative tests for malaria on 97 patients.

In all cases the agglutinated particles formed small clumps but the tests were easily read. We used artificial light for readings.

As shown in Table I, 29 (87.8%) of the 33 patients with malaria parasites in the peripheral blood gave a titer of 1:8, while 26 (78.7%) gave a titer of 1:16. Six (11.7%) of the 51 patients of groups II and IV who were regarded as non-malarious, gave a titer of 1:8; 2 (0.39%) a titer of 1:16. These data have been analyzed statistically and the differences between the two groups are significant.

The Use of Goodner's Technic. Goodner's preliminary report

TABLE I.
Results of Precipitative Tests for Malaria on 97 Patients.

Clinical group	No. of patients	No. of sera giving titer indicated							
		Below 1:8	1:8	1:16	1:32	1:64	1:128	1:256	1:512
I Malaria positive blood film	33	2	5	6	9	3	5	2	1
II Malaria excluded by clinical diagnosis and negative blood film	20	13	4	2		1			
III Malaria not excluded by clinical diagnosis but negative blood film	10			†* 3	††* 3	* 1		** 3	
IV Non-malarial febrile group. Blood films not available	31	29	2						
V Febrile conditions of undetermined origin. Blood films not available	3	1			2				

† Positive complement fixation for malaria.
* Clinically malaria. Responded to treatment.

includes a table showing results of collodion fixation in virus-antivirus systems. He combines 0.5 cc of diluted antigen, 0.1 cc of collodion particles, 0.1 cc of patient's serum and 0.3 cc saline. We have found that those quantities of reagents may be used in the precipitative test for malaria. The saline extract of *P. knowlesi* parasites has proved our most satisfactory antigen. This is prepared by grinding 0.1 g of dried parasites in saline followed by freezing and thawing for 4-6 times with dry ice and alcohol. After standing over night in the icebox the preparation is centrifuged at high speed and the supernatant used as antigen. It is not so highly colored as the alkaline extract.

We have carried out titrations of antigen and antibody in an attempt to determine the optimal ratio. Serum undiluted and diluted 1:4 in combination with antigen diluted 1:10 to 1:40 have given us satisfactory readings after an incubation period of one hour at room temperature and over night in the icebox. The collodion particles are added at the time of combining antigen and antibody. We have confirmed Goodner's finding that the collodion particles must be added at the time of combining these reagents. Negative results are obtained if the particles are added one hour later.

A control series with antigen omitted is always included. All tubes are centrifuged at 500 rpm for 5 minutes just before making the readings. Positive tests are clear-cut and are recorded at 4+ to negative.

At the present date we have records on 30 patients. Nineteen patients had malaria with positive blood films. Seventeen of these sera gave positive reactions. The 2 patients who gave negative reactions had received multiple doses of quinine. Eleven individuals gave negative blood films and malaria was not a clinical diagnosis. These individuals gave negative reactions.

Summary. Precipitative reactions in human malaria may be demonstrated (1) by the use of *P. knowlesi* antigen-coated collodion particles, or (2) by addition of collodion particles at the time of combining antigen and antibody. Macroscopic agglutination is obtained by both methods.

13408

Inhibition of Embryo Formation in Certain Nematodes by Roentgen Radiation.*

T. C. Evans, A. J. Levin and N. M. Sulkin. (Introduced by J. H. Bodine.)

From the Departments of Radiology and Zoology, State University of Iowa.

Although it is generally recognized that embryonic tissue is more radiosensitive than that of adults,[1] it was thought of interest to determine the effects of roentgen radiation on certain nematodes in which the young develop inside the body of the mother. Such experiments were undertaken in the hope that (a) they might yield information concerning differential susceptibility of cells, and (b) roentgen radiation might prove to be a useful tool in the study of trichinosis. Results of preliminary experiments[2] have been confirmed and extended in this report.

Experiments with Trichinella spiralis. Larvae, removed from infected rat muscle by pepsin digestion, were irradiated with dosages of radiation ranging from 400 r to 6,400 r (H.V.L. ca. 1.5 mm al., 427 r/m). The larvae were then fed to healthy rats and recovered from the intestine 5 days later. Microscopic study of 33 unirradiated female trichinae indicated a mean number of 59.4 worm-shaped embryos (with a standard error of the mean of 4.4) per female. Seventeen females irradiated with only 400 r contained 27.4 ± 5.5 worm-shaped embryos per animal. An irradiation of 1600 r reduced the number of worm-shaped embryos per female (25 examined) to 16.15 ±-3.25. Irradiations of 2000 r (39 examined) and 2,500 r (14 examined) completely inhibited the formation of worm-shaped embryos, but many late cleavage stages were present. Following irradiations of 3,000 r (23 examined), 3,500 r (23 examined) and 3,750 r (20 examined), embryonic development was stopped in early cleavage stages, although the adults themselves were apparently not injured. A dose of 5,000 r (6 examined) inhibited further development of the irradiated larvae.

Inasmuch as the control as well as the irradiated worms at this time contained some undeveloped embryos, the question arose as

---

* Aided by grants from the Committee on Radiations of the National Research Council, and from the Rockefeller Foundation Fund for Research on Physiology of the Cell.

[1] Bergonié, J., and Tribondeau, L., *Compt. Rend. Acad. Sci.*, 1906, **143**, 983.

[2] Levin, A. J., and Evans, T. C., *J. Parasit.*, 1940, **26**, 6, suppl. abs. No. 52, p. 31.

to their possible development, in time. Other rats were therefore fed with irradiated larvae and examined 2 months later. This allowed time for development of any viable offspring and their migration to the muscles of the host. The muscle tissue was digested and the number of worms determined by dilution counts. (In cases where there were less than approximately 2,000 worms, direct counts were made.) In Fig. 1 the numbers of worms recovered from the muscle at the end of 2 months are compared to the numbers recovered from the intestine at the end of 5 days. It can be seen from these data that the dosages required to prevent the formation of viable larvae which migrate to the muscle are much lower than those necessary to reduce the intestinal infection appreciably. Although the survival of the larvae in the muscle phase following heavy irradiation (3,500 r to 6,400 r) appears in the figure as being zero, actually from 0 to 18, (average of 7) worms were recovered. However, when one considers that the average muscle infection of rats infected with unirradiated nematodes in these experiments (controls) was 541,800, the numbers recovered from rats infected with heavily irradiated larvae are comparatively negligible. The formation of a second generation is prevented by a dosage of radiation almost 2,000 r less than that required to destroy the adults in the intestine. Therefore, it appears possible to produce, experimentally, an intestinal infection of *Trichinella spiralis* in the rat without a consequent muscle invasion.

Experiments with Rhabditis pellio. The experiments with trichinella were limited to irradiation at only one stage in the life cycle and it was impossible to follow the same individual from irradiation to the time when it should produce offspring. For this reason, experiments were performed with *Rhabditis pellio.* One of the authors[3] has cultured a pure line of these nematodes through many generations. It is therefore possible to irradiate the organisms in any stage of its life cycle and to follow the subsequent changes in the same individual.

The developmental stage selected for irradiation was that just prior to maturity. After some few preliminary experiments, it was found that results similar to those obtained with trichina could be obtained by an irradiation dose of 5,000 r. The irradiated organisms grew to maturity and some lived as much as 12 days longer than the controls. Fertilization took place but embryonic development was arrested in early cleavage stages. In later experiments, irradiated females were isolated after copulation and the number of offspring produced was observed from day to day. For example, in one ex-

---

[3] Sulkin, N. M., *Proc. Ia. Acad. Sci.*, 1940, **47**, 415.

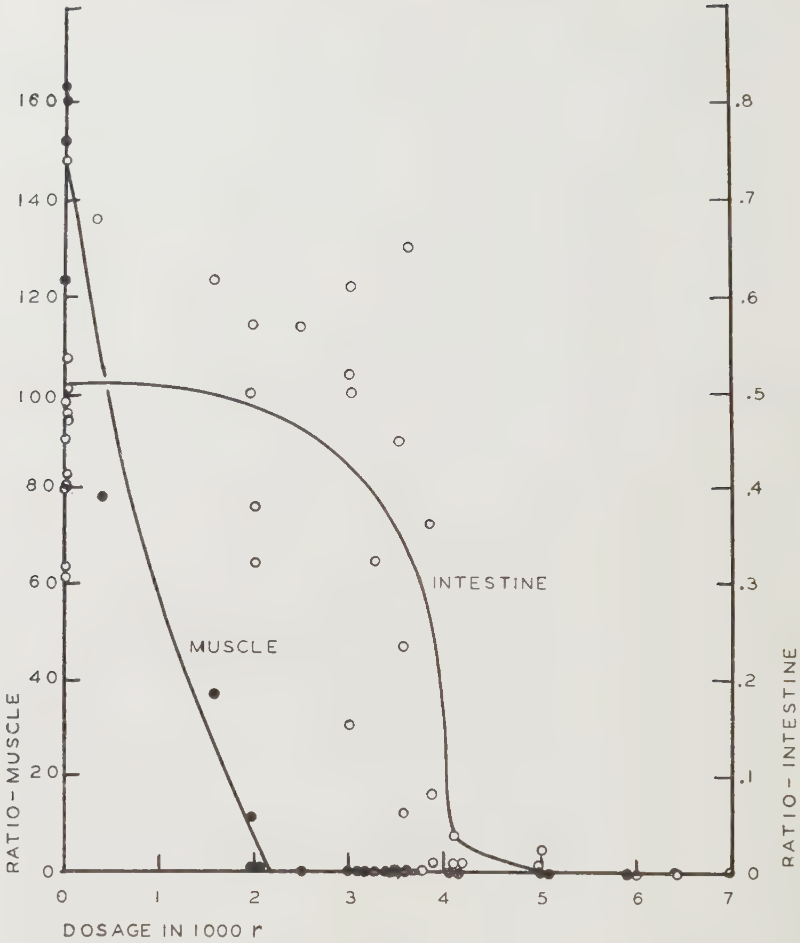


FIG. 1.

Infective larvæ of *Trichinella spiralis* were irradiated, as indicated on the abscissa, and then fed to rats. The ordinates indicate the larvæ-larvæ ratios (number of larvæ recovered divided by the number of larvæ fed). Open circles (○) indicate the larvæ-larvæ ratios of worms recovered from the intestine five days after infection, and the closed circles (●) indicate ratios of worms recovered from the muscles of rats two months after infection. The curves were fitted by inspection.

periment it was found that whereas the controls produced a total of 86.5 ± 7.95 offspring per female, the worms irradiated with a dosage of 4,928 r produced only 2.5 ± 1.5. An irradiation of 6,160 r permitted the mean production of only 0.35 offspring per female, and dosages of 7,076 r and 7,992 r completely inhibited the development of offspring in all cases. Each control and irradiated lot consisted of twenty females, in individual vials.

Discussion. The possibility of using radium radiation in the treatment of trichinosis was tested as early as 1916 by Tyzzer and Honeij.[4] They irradiated infective larvae *in vitro* and in the intestine of the host. It was found that irradiation of the larvae before feeding them to the rat reduced the number containing worm-shaped embryos when examined a few days later. Heavy irradiation of the larvae was fatal to them. Attempts to destroy the trichinae by placing radium needles in the intestine were unsuccessful. Radium radiation applied to the surface of the abdomen of infected rats failed to injure fully developed trichinae in the intestine. The irradiated worms appeared well developed and persisted longer in the intestine than did the controls. Although the experiments failed to demonstrate the therapeutic value of such radiation in the treatment of trichinosis, they indicated that the radiation appreciably modified the development of the parasite.

We can confirm the finding of the above authors, *viz.,* that irradiated adults sometimes persist longer than the controls. This was found to occur in Rhabditis when the dosage was such as to destroy the embryos but permit the adult to survive. It appears probable that the death of the female is hastened by the development of the larvae which apparently destroy the internal organs of the mother. When offspring are destroyed by radiation, the internal organs of the female remain intact and it continues to feed and grow for several days after the controls have become mere shells containing active, growing larvae.

Honess[5] found that a dosage of 800 r given to larvae before feeding them to rats lowered the resulting muscle infection to about 15% of that of the controls. This finding, that even light doses of roentgen radiation given to trichinae before feeding them to rats greatly reduces the number of offspring produced, is in general agreement with our results.

Experimental results have been obtained in these investigations which are in agreement with the recognized factors of radiosensitivity, and with results obtained by other workers in this field. We wish to suggest that it should be possible, by irradiating infective larvae of *Trichinella spiralis* with dosages of 3,000 to 4,000 r before feeding them to rats, to obtain information as to whether an intestinal infection, without the consequent muscle invasion, can produce resistance to subsequent infections of Trichinella. Investigations of this type are being carried on at present in this laboratory.

---

[4] Tyzzer, E. E., and Honeij, J. A., *J. Parasit.,* 1916, **3**, 43.

[5] Honess, R. F., *J. Colorado-Wyoming Acad. Sci.,* 1940, **2**, 44, *Biol. Abs.,* **14**, No. 14045.

Summary and Conclusions. 1. Immature forms of *Trichinella spiralis* and *Rhabditis pellio* have been irradiated with varying doses of roentgen radiation. 2. It was found that, at proper dosages, the radiation permitted the organisms to grow to maturity, to undergo copulation, and to begin the development of embryos, but the radiation killed the embryos before their development was completed. 3. Based on the foregoing data, it should be possible to ascertain whether an intestinal infection of *Trichinella spiralis,* without the consequent muscle invasion, can produce resistance to subsequent infections of Trichinella.

13409

Experimental Study of Factors Inhibiting Differentiation of Proctodeum.

A. M. SCHECHTMAN AND J. A. CANNON.

From the Department of Zoology, University of California, Los Angeles.

Introduction. It was previously reported (Schechtman[1]) that the ventrolateral blastoporal lip of the early gastrula *(Hyla regilla)* is capable of inducing a proctodeum in the belly epidermis of another embryo. The percentage of positive results was extremely variable: occasionally a group of 6 host embryos might develop an accessory proctodeum in all 6 cases; however, in most groups of 6, positive results occurred in only 1, 2, or 3 cases. This variability persisted in over 200 implantations carried out during the laying seasons of 3 consecutive years. In the entire group slightly more than 50% showed clear accessory proctodea. Accessory proctodea and tails were also produced by centrifuging late blastulae and early gastrulae (Schechtman[2]). An analysis of 983 such embryos by one of us (Cannon, unpublished) showed that only 4% were clearly positive. A striking characteristic of both implanted and centrifuged eggs is that accessory proctodea, with rare exceptions, develop on the belly region.

The aim of the present investigation was to determine the causes behind the great variability shown by the ventral blastoporal lip in its capacity to induce proctodea. The observed fact that the induced proctodea are almost always in the belly region—never adjacent to or over the axial organs—suggested that the latter may inhibit the development of proctodea.

---

[1] Schechtman, A. M., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 48.

[2] Schechtman, A. M., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 153.

Experiments. In the transplantation experiments mentioned above, the orientation of the proctodeum-inductor could not readily be controlled since the material was placed in the blastocoele. In the present experiments the proctodeum-inductor was vitally-stained and placed in a superficial position in the presumptive ectoderm of early gastrulae, so that its orientation was never in question. The experiments were as follows:

(1) Two vitally stained *dorsal lips* (which contain presumptive chorda-somite material) were transplanted, one on either side of the ventral lip of a host. The implanted dorsal lips invaginated well, so that 2 separate accessory medullary plates developed in addition to the host's own medullary plate. Sections showed that 2 notochords were developed from the dorsal lip transplants. In some cases definite accessory gut lumina were formed. *The ventral lip, surrounded by the dorsal lip transplants, invaginated and took part in the formation of the posterior abdominal region, but never formed a proctodeum.* These embryos developed no tail buds.

(1-a) It seemed possible that inhibition of the proctodeum in the above cases might be due to the experimental manipulations; therefore the following control experiments were carried out. Presumptive *ectoderm* of the early gastrula was transplanted to the same positions as in (1), namely, on either side of the ventral lip. In every case proctodea were formed from the ventral lip material. Therefore, the absence of a proctodeum found in experiment (1) is not due to mere operative procedures.

(2) A *ventral lip* was transplanted in place of the dorsal lip, so that the early gastrula possessed two ventral lips. The transplanted ventral lip became part of the neural plate, extended back into the blastopore, and formed part of the archenteric roof. When the specimen was sectioned at a later stage, it showed two neural tubes, between which lay the implanted ventral lip material which formed a wedge of mesoderm between the two nerve tubes. *There was no indication of a proctodeum* in the region of the transplant, although the host's own ventral lip did form a proctodeum.

(3) Two *ventral lips* were placed one on either side of the dorsal lip of the host, in place of the dorsolateral lips. This specimen appeared quite normal throughout. The vitally stained and transplanted ventral lips became part of the neural folds of the trunk

region, which had a perfectly normal appearance in cross sections. Again *there was no evidence of proctodeum formation* from the ventral lip transplants.

(4) Strips of tissue composed of the ventral lip (mesentoderm, the proctodeal inductor), plus some adjacent presumptive ectoderm were transplanted into two positions: (a) in such a way that in the neurula the ventral lip (mesentoderm) part of the transplant lay close to the back (axial structures) of the host, with the ectodermal part extending ventrally onto the belly region of the host. (b) Turned 180° in relation to (a) so that the ventral lip part was far down on the belly and the ectodermal part close to the back (axial organs). *In cases (a) where the mesodermal part of the transplant was closer to the back, proctodea never formed.* But when this same region was reversed so that it lay down on the belly as in (b), proctodea resulted in each case. Four cases each for (a) and (b) were observed. While this number is small, we feel the clear-cut results plus the analogous results of the other experiments (in each of which 3-4 specimens were operated upon) leave little room for doubt that the axial materials inhibit the proctodeum-forming materials.

Discussion. Bautzman[3] showed that ventral and ventrolateral lip mesoderm is less fixed or determined than dorsal lip mesoderm. Our results definitely confirm his. Thus in experiment (3) ventral lip material was converted into axial structures when placed in a new position. However, dorsal lip mesoderm as shown in experiment (1) goes on to form its normal components (somites and chorda) and induces a medullary plate in spite of its abnormal position. Bautzman worked with urodeles in which the blastoporal opening becomes the anus, and he therefore had no opportunity to notice an inhibitory effect on proctodeal inductors.

The above results suggest an explanation as to why only 50% proctodeal inductions were obtained when ventral lips were implanted into the blastocoele. Implants placed into the blastocoele are shifted about by the movements of gastrulation so that some would be carried closer to the host's axial organs. Our experiments show that implants near the axial organs would be inhibited, while those in a favorable (ventral) position would maintain their normal capacity for proctodeum induction. This would also explain the fact that secondary proctodea are nearly always found in the region of the belly rather than in the ectoderm overlying the somites or the medullary plate.

---

[3] Bautzman, H., *Arch. Ent. Mech.*, 1937, **128**, 665.

A phenomenon worthy of mention is the disappearance (dedifferentiation) of some secondary proctodea. The pigmented pit and its elevated border may regress to a flat pigmented spot which may in turn disappear. On the other hand, some induced proctodea persist and eventually communicate with the gut lumen.

Summary. Transplantation experiments show that the capacity of ventral blastoporal lips to induce a proctodeum is inhibited by the proximity of the rudiments of the axial organs. This suggests an explanation as to why, in previous experiments, proctodea were induced by only 50% of the transplants, and for the observed ventral position of accessory proctodea.

13410

Recently Isolated Strains of Pneumococci as Incitants of Experimental Pneumonia in Rats.*

W. J. NUNGESTER AND ALICE H. KEMPF.

From the Hygienic Laboratory, University of Michigan, Ann Arbor, Mich.

Although the infectivity of recently isolated strains of pneumococci has been tested both in routine injection of mice for type-identification, and in special studies,[1] the difference in ability of recovered strains to produce experimental pneumonia in animals has not been determined.

We have compared experimental pneumonic infections in rats with the clinical course in the patient from whom the pneumococci were isolated, and have correlated certain characteristics of the disease in man and rat with the type of pneumococcus involved. Cultures were obtained from 23 patients with pneumonia, and from one with otitis media during the winters of 1937-38 by Mr. Gerhardt Burde, of the Michigan State Department of Health. The pneumococci were retyped, and used in these experiments within 2 weeks of their initial isolation.

Rats were inoculated intrabronchially in the left lobe of the lung with 10^{-5} cc of cultures of these organisms suspended in sterile mucin, according to a method previously described.[2] Surviving

---

* These studies were aided by a grant from the Horace H. Rackham School of Graduate Studies.

[1] Rosenau, M. J., Felton, L. D., and Atwater, R. M., *Am. J. Hyg.*, 1926, **6**, 463.

[2] Nungester, W. J., and Jourdonais, L. F., *J. Infect. Dis.*, 1936, **59**, 258.

TABLE I.

Correlation of Clinical Pneumonia with Experimental Pneumonia Produced in Rats, Using Strains of Pneumococci Obtained from Patients.

Case No.	Type	Clinical course of patient	Number of rats						"Toxin" tested in mice; mld in g†
			Inoc.	Indicated percent of lobe involved			Marked pleurisy	Died	
				90-100%	25-90%	0-25%			
33	1	Mild	2	2			1	1	.030
22	1	Moderate	3	2			3	0	.004
30	1	”	2	1		1	0	0	.030
23	1	Severe	3	3			3	1	.060
39	1	”	2	1		1	0	0	.030
34	1	” D*	3	2	1	1	1	0	.030
27	1	(Otitis media) D	2	2			1	1	.030
		Total	17	13 (76%)	1	3	9 (53%)	3 (18%)	Avg .031
50	2	Mild	3	2	1		2	3	.020
31	2	Moderate	2	2			0	2	.030
28	2	Severe	2	1	1		1	2	.010
26	2	” D	2	1	1		1	2	.015
49	2	” D	2	2			1	2	.030
14	2	” D	3	2	1		0	3	.030
		Total	14	10 (72%)	4		5 (36%)	14 (100%)	Avg .023
9	3	Severe	3	2	1		1	2	.020
37	5	”	3	1	1		0	0	.005
12	6	”	3	1		1	0	1	.030
18	6	”	3	3			0	0	.010
7	7	”	3	2		1	1	0	
10	10	Mild	3	3			2	3	.020
32	13	”	3	3			0	0	.030
13	15	Severe D	3	3		1	0	0	.015
16	19	”	3		2	1	0	0	.030
36	19	Moderate	2	2			0	0	.005
8	27	Mild	3	2		1	0	1	.030
		Total	32	22 (69%)	4	5	4 (13%)	7 (22%)	Avg .019

*D—Patient died.

†The toxins were prepared and tested by Burde, Barret, and Glassen. The authors are grateful for the privilege of including this material.

animals were etherized on the seventh day after inoculation. All animals were necropsied, and gross changes were observed.

In Table I the severity of the disease in the patient, as well as in the experimentally inoculated rats, is indicated. The number of animals showing varying degrees of consolidation of the infected lobe, the number having marked pleurisy, and the number that died are recorded. Also, an estimation is given of the minimal lethal dose of "toxins" produced from the same strains of pneumococci by Burde, Barret and Glassen,[3] according to the procedure described by Dick and Boor.[4]

It will be noted that in the group of 17 rats infected with Type 1 pneumococci, the mortality was 18%, whereas in a group of 14 animals infected with Type 2 strains, the mortality was 100%. This difference parallels the severity of the disease in the patients from whom the organisms were isolated. Further, we have been informed by Dr. Alvin Price that among the patients with lobar pneumonia at Receiving Hospital, Detroit, during the winters of 1937-38, a mortality of 19% was noted in the 42 patients with Type 1 pneumonia, while among the 68 patients with Type 2 infections, the mortality was 35%. Thus the differences observed in the rats are seen to be in agreement with the clinical findings in this geographical region during that period. The mortality of rats inoculated with available strains of other types of pneumococci was about 22%.

The incidence of pleurisy was somewhat higher in Type 1 than in Type 2 infections. This was in contrast to the results already discussed on the difference in mortality produced by the two types, and may be accounted for by the fact that the average survival of rats that died was 4.6 days in the Type 2, and 2.8 days in the Type 1 group. Further, 82% of the animals inoculated with Type 1 pneumococci were etherized after surviving for 7 days. None in the Type 2 group lived for this length of time. It has been suggested that at least 2 or 3 days are required for the development of marked pleurisy in experimental lesions in rats.[2] Nevertheless, the development of pleurisy is dependent in part on the organism. This is indicated by the fact that a very low incidence (13%) of pleurisy was noted in the group of rats inoculated with strains of pneumococci Types 3 to 27. In this group, 78% of the animals survived until sacrificed at 7 days. The average survival of these animals that died was 3.7 days.

These factors, time of survival of the rat and strain of pneumococcus, which affected the spread of infection to the pleura, did not control the spread of the pneumonic process within the pulmonary parenchyma, however. It will be noted that the incidence of involvement from 90 to 100% of the inoculated left lobe in the 3 groups of animals, Type 1, Type 2, and Types 3 to 27, was approximately the same.

Histological sections from the lungs of 6 animals infected with Type 1, 5 with Type 2, and 8 with other types, were made and examined for the amount of edema in the tissue, the amount of red-cell infiltration into alveoli, the number of alveoli completely filled with cellular exudate, necrosis of pulmonary parenchyma, and the severity of bronchitis. No significant differences could be attributed to the type of pneumococcus producing the experimental lesion. It is not advisable to place too much emphasis on the histopathology because of the limited number of infected lungs studied.

---

[3] Personal communication.

[4] Dick, G. F., and Boor, A. K., *J. Infect. Dis.*, 1937, **61**, 228.

There was complete lack of agreement in the titer of "toxins" as prepared by Burde, Barret and Glassen[3] and the severity of the experimental lesions. For example, in the third group of rats, seen in Table I, the mortality of those inoculated with pneumococci which yielded a stronger than average "toxin" was 0%; for the strains producing a weaker than average "toxin", 16%; and the mortality produced by organisms with toxins having mld's equal to the average mld was 83%.

13411

Influence of Lactogenic Preparations on Mammary Glands and Time of Vaginal Opening in Young Rats.*

Wm. R. Lyons, M. E. Simpson and H. M. Evans.

From the Institute of Experimental Biology and Division of Anatomy, University of California.

In studying the cycle-inhibiting action of lactogenic hormone in the rat, it has been of interest also to observe changes brought about indirectly by this hormone on the mammary apparatus. Previously, it had been shown[1] that crude anterior lobe extracts (now known to have a high lactogenic hormone content) caused lobular mammary development in normal but not oöphorectomized rats. This suggested an indirect stimulation of the mammary gland by a pituitary factor acting possibly through the luteal tissue often induced in excess by such extracts. More recent work[2] on the hypophysectomized rat has indicated that whereas the follicle-stimulating and luteinizing

---

* Aided by grants from the Research Board of the University of California and from the Rockefeller Foundation. Assistance was rendered by the Works Projects Administration, Official Project No. 65-1-08-62, Unit A-5.

[1] Evans, H. M., and Simpson, M. E., *Am. J. Physiol.*, 1931, **98**, 511.

[2] Evans, H. M., Simpson, M. E., Lyons, W. R., and Turpeinen, K., *Endocrinol.*, 1941, **28**, 933.

(I.C.S.H.) fractions are essential for the production of corpora, these structures are activated and secrete progestin under the influence of lactogenic hormone. Astwood[3] has found corpus-luteum-activating substances in the rat placenta (cyonin) as well as in the anterior pituitary (luteotrophin). He has shown that lactogenic preparations made according to the method used in this laboratory did activate the corpora lutea, but did not maintain or produce lobule growth in the mammary gland of the hypophysectomized rat. On the other hand, crude rat placental extracts, whole pituitary powders, or crude luteotrophin did produce extensive lobular proliferation.

In the experiment reported herein, 3 daily levels of lactogenic hormone (0.5, 1.0, and 2.0 mg) were given subcutaneously from day 21 to day 50 to 3 groups of 6 rats. Six saline-injected rats served as controls. Animals of this age were used in order to determine whether lactogenic hormone injected into prepuberal rats would inhibit the pituitary gonadotrophins and thus delay the time of vaginal opening.

The data in Table I show that little difference in the time of vaginal opening was observed between the control group and the groups injected with hormone. The average time for 5 of the control rats was 43 days and for the injected groups, 38, 39 and 35 days respectively for the animals receiving 0.5, 1.0, and 2.0 mg levels of lactogenic hormone. Thus it might be said that the time of first vaginal opening was slightly advanced in the lactogenic groups and certainly was not delayed. This finding indicates that lactogenic hormone does not inhibit the output of F S H by the immature rat's pituitary. It should be emphasized, however, that although sexual maturation was not delayed, the oestrous cycles thereafter, especially in the higher dose groups (Table I), were definitely inhibited. The initial crop of corpora lutea had persisted in most of the animals for the duration of the experiment and no new follicular maturation had taken place. This finding, combined with the double evidence for progestin secretion, namely, elaborate vaginal mucification and lobular mammary growth suggests that the inhibition of pituitary F S H secretion may depend upon a certain level of progestin in the blood stream.

Mammary spreads were taken from each rat, fixed in formol and stained *in toto* with alum-carmine. The glands of the control rats as well as those from animals in which the cycle was not inhibited showed little more than a duct system with occasional alveolar-bud formation (Fig. 1). Glands from all of the animals in which the

---

[3] Astwood, E. B., *Endocrinology*, 1941, **28**, 309.

TABLE I.
Influence of Lactogenic Hormone on Time of Vaginal Opening and on Mammary Gland of Normal Young Rats.

Rat	Treatment	Day of vaginal opening	Vaginal cycle	Mammary gland
W 27	.5 cc saline daily	42	Regular	D*
W 29	30 days, subc.	44	”	D
BH 37		closed 51	None	D
BH 44		39	Regular	D
BH 76		46	”	D
B 91		45	”	D
	Avg (5)	43		
GH 03	.5 cc (.5 mg) lactogenic	42	Not inhibited	D
G 12	hormone daily, 30 days,	34	Second oestrus on day 47	LA†
GH 39	subc.	38	Inhibited after open.	LA
GH 56	25-30 I.U./mg	37	” ” ”	LA
B 78		38	Regular	D
B 89		37	Inhibited after open.	LA
	Avg	38		
GH 02	.5 cc (1 mg) of same	37	Inhibited after open.	LA
W 10	lactogenic hormone	44	Opened too late	D
BH 36		40	Inhibited after open.	LA
B 82		38	” ” ”	LA
BH 93		40	” ” ”	LA
BH 97		34	Regular	D
	Avg	39		
W 14	.5 cc (2 mg) of same	34	Inhibited after open.	LA
BH 43	lactogenic hormone	33	” ” ”	LA
B 80		38	” ” ”	LA
B 88		39	” ” ”	LA
W 94		34	” ” ”	LA
BH 96		33	Second oestrus on day 47	LA
	Avg	35		

*D—Duct development with sparse alveolar budding (Fig. 1 left).

†LA—Lobule-alveolar development (Fig. 1 right).

Note: Where the vaginal cycle was inhibited a mucified smear was found and a high degree of mucification was noted in sections of vagina. In these animals only one "crop" of corpora lutea was found in the ovaries, whereas in rats showing cycles more than one crop was in evidence. As previously reported,[2,4] 0.5 mg is the border-line dose of lactogenic hormone capable of maintaining already existing corpora lutea and preventing normally recurring oestrous cycles in adult rats.

cycle was completely inhibited after the first vaginal opening showed excellent lobular development[†] (Fig. 1), due apparently to the combined action of estrin and progestin.

[†] Thus a statement made recently[4] in connection with earlier work by one of us (W.R.L.) would seem to be in error. In testing for growth hormone, 10 mg of lactogenic hormone had been given to normal rats daily for 20 days. Only gross observations were made at autopsy and the mammary development was dismissed as negligible because it did not compare with that of a rat at the end of pregnancy.

[4] Evans, H. M., Simpson, M. E., and Lyons, W. R., Proc. Soc. Exp. Biol. and Med., 1941, **46**, 586.

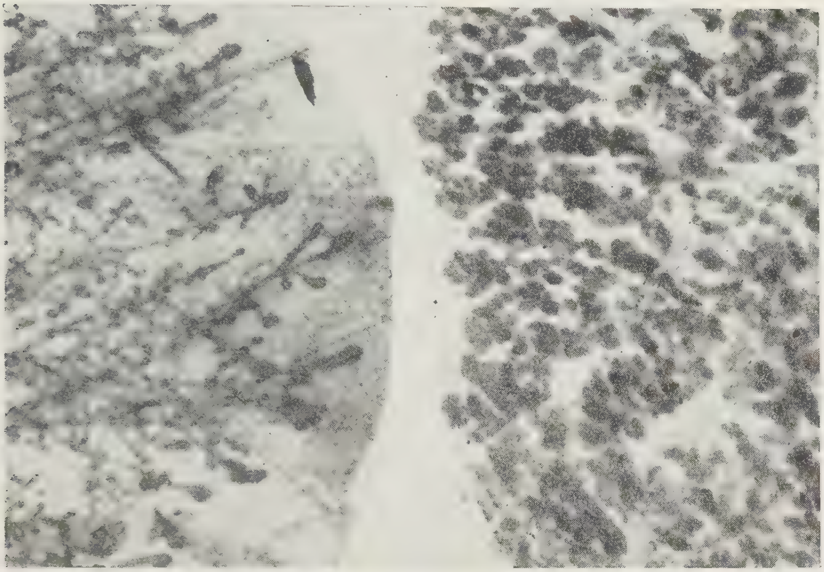


FIG. 1.

Left: Mammary gland from control rat W 27 injected daily from day 21 until day 50 with saline.

Right: Same from rat W 94 injected daily from day 21 until day 50 with 2 mg lactogenic hormone.

Note complete lobular development. Magnification × 10.

Summary. Lactogenic hormone in doses from 0.5-2.0 mg given subcutaneously daily beginning at day 21 to rats did not delay, but in some cases may have slightly advanced the day of vaginal opening. Continued injection of the same doses of hormone thereafter resulted in continuous vaginal mucification for the duration of experiment (approximately two weeks) and in lobular development of the mammary gland.

13412

Poliomyelitis Virus from Flies.*

JOHN A. TOOMEY, WILLIAM S. TAKACS AND LINDA A. TISCHER.

From the Division of Contagious Diseases, City Hospital, and the Department of Pediatrics, Western Reserve University, Cleveland, O.

Flexner and Clark[1] and Howard and Clark[2] have demonstrated

---

* Sponsored by The National Foundation for Infantile Paralysis, Inc.

[1] Flexner, S., and Clark, P. F., *J. A. M. A.*, 1911, **56**, 1717.

[2] Howard, C. W., and Clark, P. F., *J. Exp. Med.*, 1912, **16**, 850.

that artificially contaminated house flies can carry the virus of poliomyelitis. Rosenau and Brues[3] and Anderson and Frost[4] allowed stable flies to bite poliomyelitis-infected monkeys first and then non-infected animals, which subsequently came down with poliomyelitis. Rosenow, South and McCormack[5] were able to produce the disease by injecting filtrates of flies collected in epidemic areas, although the disease was not transferable to other monkeys. Recently, Paul, Trask and associates[7] isolated virus from flies obtained from two widely separated areas—Connecticut and Alabama.

During the past 10 years, 20 experiments were done in this laboratory to recover poliomyelitis virus from house flies, all of which were unsuccessful. Dead flies were washed and the suspension filtered. The filtrate was injected either intracerebrally or intranasally, or both, into *M. mulatta* monkeys. A few experiments with filtrates of ground stable flies were also unsuccessful.

Although the disease morbidity decreases after the first frost and the fly population is minimal at the same time, and although virus might be found in or on flies during large epidemics, these facts seemed to be of slight epidemiologic importance because: (1) the virus could not be consistently recovered from these insects during either epidemic or non-epidemic years; (2) the disease did not always occur in crowded communities infested with flies, but more often in clean surroundings devoid of flies; (3) the peak of the disease and that of the greatest fly population did not correspond.[6] It was thought, nevertheless, that it should be possible to isolate the virus from some flies, especially from those which in epidemic areas would become grossly infected. Thus, if flies were selected from areas contaminated with sewage, positive results might be obtained.

Since virus material might be absent or present in subinfective doses in one or two flies, it was decided to work with large numbers of these insects. Fly traps were set near the mouth of a brook that emptied raw sewage into Lake Erie. Excrement was plainly visible on the top of the water. This brook drained parts of the northeastern section of the city where the epidemic first appeared this year—an area in which a large number of cases were found. A large yield of flies was obtained, mostly large, blow flies with only an occasional small house fly and a few wasps and moths.

---

[3] Rosenau, M. J., and Brues, C. T., *Trans. XV Internat. Cong. Hyg. and Demog.*, Washington, 1912, 1913, **1**, 616.

[4] Anderson, J. F., and Frost, W. H., *U. S. Pub. Health Rep.*, 1912, **27**, 1733.

[5] Rosenow, E. C., South, L. H., and McCormack, A. T., *Kentucky Med. J.*, 1937, **35**, 437.

[6] Johnson, G. A., *J. Am. Water Works A.*, 1916, **3**, 249, 857.

[7] Paul, J. R., Trask, J. D., Bishop, M. B., Melnick, J. L., and Casey, A. E., *Science*, 1941, **94**, 395.

About 2,000 etherized flies were put in a glass bead grinder, 200 cc of sterile distilled water added, the whole ground to a homogeneous fluid and then centrifuged. To the supernate was added 15% ether and the mixture was left in the icebox over night. The next morning the suspension was poured into a large Visking casing, which was hung between electric fans, and it dried to a residue in from approximately 72 to 96 hours. The latter was then suspended in 120 cc of normal physiologic saline and 15% ether was added; the next day the suspension was tested for sterility. The mixture was still contaminated, and it was again treated with ether. When it was sterile, the ether was drawn off from the suspension under a partial vacuum.

Two cc of the suspension was injected intracerebrally into a *M. mulatta* monkey, 0.5 cc was instilled into each nostril daily for 5 days, and 35 cc intraperitoneally. The intraperitoneal injection was repeated on the fourth day.

This monkey developed muscle weakness on the seventh and unmistakable poliomyelitis on the tenth day. It was sacrificed. Another animal injected in a similar manner also developed the disease. A 10% suspension of the cord of the first animal sacrificed was injected into a third monkey, and the disease was produced.

A second isolation has been made from flies collected in an outhouse. Sabin and Ward[8] have also isolated virus from flies collected this season from 3 urban areas—2 in Cleveland, Ohio, and one in Atlanta, Georgia.

13413 P

Hereditary Predisposition to Sensitization in Guinea Pigs.*

J. L. Jacobs, J. J. Kelley and S. C. Sommers.

From the Department of Bacteriology and Immunology, Oglethorpe University School of Medicine, Georgia, and the Department of Pathology and Bacteriology, Tufts College Medical School, Boston, Mass.

Blocking experimental studies on the inheritance of allergy has been lack of a sensitizing agent which would discriminate sharply between individuals within a species. Chase[1] showed that it was possible to set up lines of guinea pigs of different susceptibilities toward 2:4 dinitrochlorobenzene. However, crosses between members of the more and less susceptible colonies were not undertaken because the latter group always contained susceptible individuals, and thus the conditions for a precise genetic analysis were lacking. Some years ago we also attempted to breed out a guinea pig strain which was not susceptible to sensitization with 2:4 dinitrochlorobenzene, and meeting with similar difficulties, thought it advisable to search for a less universal sensitizing agent, which discriminated more sharply between susceptible and resistant animals. Allyl isothiocyanate was tried because, although Landsteiner and DiSomma[2] had attempted to sensitize guinea pigs with this substance without success, they had found that one of 6 humans treated became hypersensitive, a situation favorable to heredity work. It was not deemed advisable to attempt large scale sensitization experiments on humans with this substance since mustard is a common article of diet, and therefore a new attempt was made to sensitize guinea pigs.

---

[8] Sabin, A. B., and Ward, R., personal communication.

* This investigation was aided by a grant from the Charlton Research Fund of Tufts College Medical School.

Treatments with allyl isothiocyanate were carried out by applying one drop of mustard oil to the back, after clipping the hair, twice a week for 3 to 5 weeks. Tests were performed at least 2 weeks after the last treatment by spreading one drop of a 5% solution of mustard oil in dioxane on the flank after removing hair with a clipper, followed by treatment with an electric shaver before reading on the following day.

Of 57 albino guinea pigs treated and tested in this way, 6 showed moderate to strong reactions and 5 others were weakly positive. A typical experiment with 12 pigs is shown in Table I, Column A.

Further treatment had comparatively little effect in increasing intensity of sensitization or the proportion of hypersensitive animals.

In a similar but small scale experiment with alpha naphthyl isothiocyanate, with treatments of one drop of a saturated acetone solution spread on the back, some evidence of sensitization was observed. With phenyl isothiocyanate sensitization effects were very slight or negative and methyl, isopropyl, and n-butyl thiocyanates gave negative results. Because of the occasional nature of sensitization in this group, small scale negative results necessarily carry little weight.

Selective breeding experiments carried out over a period of almost 3 years and involving 4 generations of the animals most readily sensitized to allyl isothiocyanate have apparently succeeded in pro-

---

[1] Chase, Merrill W., *J. Exp. Med.*, 1941, **73**, 711.

[2] Landsteiner, K., and DiSomma, A. A., *J. Exp. Med.*, 1938, **68**, 509.

TABLE I.

Combined table. All readings were made on the day following the application. Untreated controls and animals before treatment with allyl isothiocyanate were all negative.

A—Unselected guinea pigs		B—Offspring of improved strain	
No. of pig	Animals treated with allyl isothiocyanate	No. of pig	After treatment with allyl isothiocyanate
1	Negative	13	Faintly pink
2	ʼʼ	14	Pale ʼʼ
3	Almost negative	15	Faintly ʼʼ
4	Pale pink	16	Pale ʼʼ
5	Negative	17	Pink
6	ʼʼ	18	Pale ʼʼ
7	ʼʼ	19	ʼʼ ʼʼ
8	ʼʼ	20	ʼʼ ʼʼ
9	ʼʼ	21	ʼʼ ʼʼ
10	Faintly pink	22	Pink
11	Almost negative	23	ʼʼ
12	Negative	24	Pale ʼʼ

ducing a stock practically all of which are readily and strongly sensitized to mustard oil, as illustrated by the combined test performed on the members of several litters which is given in Table I, Column B.

A comparison of Columns A and B, shows that the increased reactivity of the latter group as compared with unselected animals is very striking, after sensitization. No increased reactivity to the 5% dioxane solution before sensitization was observed in the improved strain. Cross tests with animals sensitized to 2:4 dinitrochlorobenzene showed that the sensitization was specific. Thus segregation of an hereditary character determining capacity for sensitization by this substance seems to have been accomplished. A strain of pigs resistant to sensitization is being bred out for heredity studies. This effort has considerable hope of success, since most unselected guinea pigs cannot be sensitized to allyl isothiocyanate. Other questions, such as whether increased capacity for sensitization is general or specific, are being examined.

Summary. Sensitization to allyl isothiocyanate was accomplished in a small percentage of unselected guinea pigs. By selective breeding a strain was obtained practically all the members of which could be readily and strongly sensitized with this substance. A strain resistant to sensitization is being further bred out.

13414

Effect of Oral and Parenteral Administration of Vitamin E on Creatinuria and Symptoms of Dystrophic Rabbits.*

C. G. Mackenzie and E. V. McCollum.

From the Department of Biochemistry, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Md.

Nutritional muscular dystrophy in the rabbit may be cured or prevented by the oral administration of d,l-α-tocopherol[1] (synthetic vitamin E). Mattill[2] has stated that the acetate of α-tocopherol is much less effective when given parenterally or subcutaneously than by mouth, and Eppstein and Morgulis[3] have reported that the intramuscular injection of the acetate in olive oil at 5 or 10 mg levels failed to cure dystrophic rabbits. According to Knowlton, *et al.*,[4] the injection of the ester was more effective in curing the relatively mild symptoms of dystrophy in older rats than in preventing them in young animals. It was shown by Demole[5] that 20 times the active oral dose of α-tocopherol acetate was ineffective when injected in preventing resorption in E-deficient female rats. On the other hand, the unesterified vitamin when injected showed one-fifth to one-tenth of its oral activity. Goettsch and Pappenheimer[6] have recently reported a somewhat higher activity for injected E in the rat. We wish to report the results of injecting dystrophic rabbits with α-tocopherol[†] (in a pure form or in peanut oil solution) and with the water-soluble sodium salt of the phosphoric acid ester of α-tocopherol.[7]

Since we have previously shown that an abnormal creatinuria (and extensive muscle lesions) may exist in actively growing chronically E-deficient rabbits in the absence of overt symptoms,[8] urinary

---

* Supported by grants from the Research Corporation of New York and the Carnegie Institution of Washington.

We are indebted to Jeanette Nickles for technical assistance.

[1] Mackenzie, C. G., and McCollum, E. V., *J. Nutrition*, 1940, **19**, 345.

[2] Mattill, H. A., *J. Nutrition*, 1940, **19**, Proc. Am. Inst. Nutr., p. 13.

[3] Eppstein, S. H., and Morgulis, S., *J. Nutrition*, 1941, **22**, 415.

[4] Knowlton, G. C., Hines, H. M., and Brinkhous, K. M., Proc. Soc. Exp. Biol. and Med., 1939, **42**, 804.

[5] Demole, V., *Ex. Compt. Rend. de la Société des Physiologistes Suisses*, 1939, July.

[6] Goettsch, M., and Pappenheimer, A. M., *J. Nutrition*, 1941, **22**, 463.

[†] Kindly supplied by Merck and Company, Inc.

[7] Karrer, P., and Bussmann, G., *Helv. Chemica Acta*, 1940, **23**, 1137.

[8] Mackenzie, C. G., Levine, M. D., and McCollum, E. V., *J. Nutrition*, 1940, **20**, 399.

creatine and creatinine were determined daily, resulting in the amplification and extension of earlier observations on the excretion of these substances in dystrophic and cured animals. Furthermore, the production and symptomatology of an extremely severe dystrophy of several months' duration is reported.

The methods employed in these experiments have been described elsewhere.[1, 8] The terms "creatine" and "creatinine" are used to designate chromogenic substances in urine giving a positive Joffé reaction according to the procedure of Folin.[9] Creatine is expressed as milligrams of creatinine. All of the animals used had been cured of one previous attack of dystrophy by the oral administration of α-tocopherol.

Sixteen young rabbits on diet 13 were allowed to develop a daily creatinuria of 60 to 70 mg. At that time they weighed approximately 1000 g. Eight were injected intramuscularly or subcutaneously with 20 mg of d,l-α-tocopherol in 0.2 cc of peanut oil, 5 received the same dose orally, and 3 were untreated. The untreated animals died in 8, 9, and 10 days after losing 185, 80, and 380 g. One of the injected animals died in 7 days, and was omitted from the group. The other 7 were alive at 12 days, although the physical symptoms of dystrophy had grown progressively worse. Food consumption, however, remained at the preinjection average of 20 g daily, and the sharp decline in weight usually associated with such severe physical symptoms did not occur. The average loss in weight for the 12-day period was 100 g. Although the oral administration of the same dose of α-tocopherol resulted in the disappearance of physical symptoms within 7 days, and a growth response in 2 days (with an average gain of 250 g during 12 days), the results of this experiment suggest that the injected tocopherol was not altogether without effect in prolonging life, and stabilizing food consumption and weight.

A comparison of the creatine and creatinine excretion of these two groups is illustrated in Fig. 1. The creatine excretion of the 3 untreated animals is also included. The consistently high creatine excretion of the injected animals is in contrast to the rapid drop of urinary creatine in the group receiving the same dose of α-tocopherol orally. The creatine excretion curve of the latter group demonstrates the value of daily creatine determinations in measuring the level of vitamin E nutrition. During the last 3 days of the experiment, while the creatine was rising, the average daily gain in weight was 25 g, and there were no physical symptoms. Yet from the

---

[9] Folin, O., *J. Biol. Chem.*, 1914, **17**, 469.

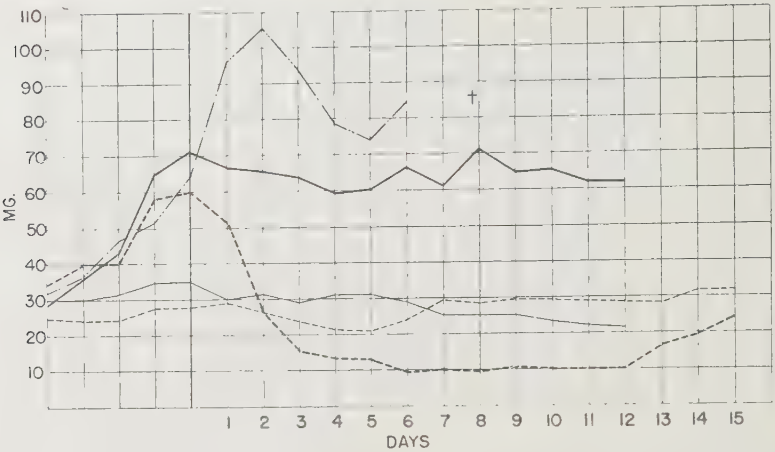


FIG. 1.

Average daily creatine and creatinine excretion of dystrophic rabbits receiving 20 mg of d, 1-α-tocopherol in 0.2 cc of peanut oil orally or parenterally.

The time of treatment is indicated by the thickened vertical line at 0 days. The 4 previous days are not numbered. The uppermost line (long dash) represents the creatine excretion of 3 untreated animals, and † indicates the approximate time of their death. The heavy and thin unbroken lines represent, respectively, the average creatine and creatinine excretion of 7 injected rabbits. The heavy and thin broken lines represent, respectively, the average creatine and creatinine excretion of 5 rabbits treated orally.

point of view of adequate nutrition, a deficiency of the vitamin had again set in, and it is likely that once more muscle lesions had developed.[8]

Of interest is the fall in urinary creatinine in the injected animals beginning on the seventh day. This was to be expected on the basis of Bloch and Schoenheimer's[10] observation that urinary creatinine in normal rats represents 2% of the body creatine. Our previous failure to detect an unmistakable decrease in creatinine excretion was probably due to the earlier death of most of our dystrophic rabbits. The temporary fall in creatinine, paralleling the fall in creatine in animals responding to vitamin E, has already been noted.[1]

We were prompted by the results of this experiment to try the effect of injecting larger amounts of d,l-α-tocopherol. Two dystrophic rabbits were injected with 100 mg and 2 with 200 mg of pure α-tocopherol, and 2 with 200 mg of α-tocopherol in peanut oil. One animal of each pair was injected intramuscularly and the other subcutaneously. The site of injection and the form of the preparation were apparently unimportant. Two of the 4 animals receiving 200 mg responded in an uneventful albeit somewhat slow fashion, 12 days being required for the creatine to reach 10 mg as compared with

---

[10] Bloch, K., and Schoenheimer, R., *J. Biol. Chem.*, 1939, **131**, 111.

5.6 days for the orally treated animals (Fig. 1). However, the other 2 animals receiving 200 mg behaved in a more interesting fashion as did one of those receiving 100 mg. (The other rabbit on the latter level died in 17 days.) These 3 rabbits started to gain weight one week after injection. Simultaneously the urinary creatine (70 to 90 mg) began a gradual decline which terminated at a level of 20 to 40 mg on about the 38th day and then began to rise again. The urinary creatinine began to rise slowly after 20 days. One of the animals was killed at 41 days, with a rising urinary creatine and creatinine. At that time it had gained a total of 550 g and was still growing.

The other 2 rabbits were continued. Urinary creatine rose to 65 mg by the 58th day. It was accompanied by an increase in the creatinine excretion to 45 mg daily. The creatinine then slowly declined from that level to 5 mg by the 75th day. There was a concomitant fall in creatine excretion in both animals. Rabbit No. 311 developed diarrhea, lost weight for 3 days, and died on the 80th day. The daily creatine excretion at this time averaged 15 mg. Rabbit No. 307 developed the same symptoms and died on the 95th day. Its creatine excretion for the last few days of life averaged 25 mg. During the entire experimental period both rabbits exhibited severe symptoms of dystrophy. This was particularly so of No. 307, whose death was predicted as early as the second week of the experiment. From that time on it was practically prostrate. Nevertheless, it gained 300 g between the 30th and 92nd day. Rabbit No. 311 gained 1000 g during the experiment excluding the 3 days before death.

The microscopic picture of the thigh muscles of these growing dystrophic animals was most interesting. There was extreme fatty infiltration, a moderate increase in connective tissue, and severe atrophy of the remaining muscle fibers. Some of these atrophic fibers showed hyalinization and necrosis, indicating that the active degeneration characteristic of all stages of E deficiency in the rabbit had not ceased. Similar changes in guinea pigs have been described by Goettsch and Pappenheimer.[11] The large numbers of fat cells explains in part why these animals were gaining weight while their muscle fibers were undergoing necrosis or atrophy. This picture was in sharp contrast to that found in growing chronically E-deficient rabbits which exhibit creatinuria, but no physical symptoms.[8] Although their muscle fibers showed hyalinization and necrosis, repair was apparent, and neither fatty infiltration nor marked atrophy was observed.

---

[11] Goettsch, M., and Pappenheimer, A. M., *J. Exp. Med.*, 1931, **54**, 145.

In an endeavor to throw some light on the poor and variable response to the injection of massive doses of α-tocopherol, a finding in harmony with Demole's results in rats,[5] we prepared the water-soluble sodium phosphate of α-tocopherol according to the procedure of Karrer and Bussmann.[7] Since the purity of our preparation was not determined, the doses employed represent maximum values only. Aqueous solutions containing the equivalent of 20 and 30 mg of α-tocopherol were injected subcutaneously into 2 dystrophic rabbits. The high urinary creatines of the 2 rabbits fell to 10 mg daily in 8 and 9 days respectively. There was a prompt growth response and the physical symptoms disappeared within a few days. This suggests that the activity of injected vitamin E in rabbit dystrophy, as in rat sterility,[5, 7] is determined largely by its physical state.

Conclusions. The oral administration of 20 mg of α-tocopherol to dystrophic rabbits produces a rapid fall in the urinary creatine. Following the parenteral administration of the same dose, the creatine remains at a high level, and the creatinine decreases. The injection of massive doses of α-tocopherol cures dystrophy in some cases, while in others it extends life and promotes growth for several months without curing the symptoms of the disease.

13415 P

Clinical Fetal Electrocardiography. Its Practical Accomplishment.

ARTHUR J. GEIGER, WILLYS M. MONROE AND ALLAN V. N. GOODYER. (Introduced by Francis G. Blake.)

From the Department of Internal Medicine, Yale University School of Medicine.

The recording of the electrocardiogram of the human fetus *in utero*, although long attempted (Cremer,[1] Foá[2]), has been only indifferently successful even late in pregnancy.[3, 4, 5]

We have developed a technic of definite clinical value by using a single stage resistance-coupled amplifier (successfully employed[6] on the embryo chick heart) with a conventional portable electrocardiograph. The baseline of the tracing was stabilized by means of series condensers of 0.001 to 0.05 microfarads capacities which were coupled to the lead wire when needed for damping the respiratory artefacts of the mother. The fetal heart potentials, picked up by disc electrodes on the pregnant subject's abdomen (xiphoid-pubis application to minimize the pickup of the maternal electrocardiogram) were amplified 20 times, thereby bringing the fetal electrocardiogram into plain view while keeping that of the mother within the limits of recording of the apparatus. The amplifier measures 9 x 5 x 7 inches, weighs 12 pounds, is constructed of standard radio parts, is simple to operate, and together with the electrocardiograph makes an easily portable device.

---

[1] Cremer, M., *München med. Wchnschr.*, 1906, **53**, 811.

[2] Foá, C., *Giornale della R. Acad. di Med. di Torino*, 1911, **4**, 90.

[3] Strassmann, E. O., *Proc. Staff Meet. Mayo Clin.*, 1938, **13**, 251.

[4] Bell, G. H., *J. Obst. and Gyn. Brit. Emp.*, 1938, **45**, 802.

[5] Mann, H., and Bernstein, P., *Am. Heart J.*, 1941, **22**, 390.

With this technic we are able to record the action current of the fetal heart in virtually all instances during the last 2 months of pregnancy, usually in the sixth and seventh month, and frequently in the fourth and fifth month. Fig. 1 illustrates a typical result, and Fig. 2 shows one of our best.

As is obvious from the illustrations, the baseline irregularity is the present limiting factor, for it tends to obscure the characteristically small fetal electrocardiogram of early pregnancy. These artefacts

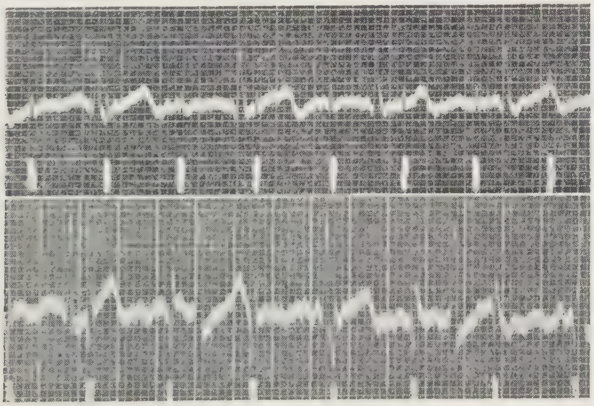


Fig. 1 (above). Fig. 2 (below).

Fig. 1. Tracing typical of those usually obtained. Fetal electrocardiograms indicated by marks at 0.39 sec. intervals (fetal heart rate 154/min.). Pregnancy in 21st week before term.

Fig. 2. Tracing obtained in 5th month of pregnancy. Fetal electrocardiograms indicated by marks at 0.42 sec. intervals (fetal heart rate 143/min.).

---

[6] Hoff, E. C., Kramer, T. C., Du Bois, D., and Patten, B. M., *Am. Heart J.*, 1939, **17**, 470.

probably arise from fibrillary movements of muscle and from external electrical phenomena; experiments to determine their nature and to eliminate them are under way.

The technic permits the prompt differential diagnosis of pregnancy from other abdominal tumors, it is free from false positive results, and it is less time consuming than biological tests for pregnancy. A positive result is certain proof of life of the fetus. Finally, the detection of twin pregnancy becomes possible earlier than by any other method of examination.

We believe that clinical fetal electrocardiography is now a practical laboratory accomplishment and represents a useful clinical aid.

We are grateful to Mr. H. Harwell and Mr. R. K. Hellman of the Connecticut Telephone and Electric Corporation of Meriden, Conn., for the amplifier employed in these experiments.

13416

Influence of Cell Concentration on Respiration Rate of Sperm.*

C. F. WINCHESTER AND FRED F. MCKENZIE.

From the Animal Husbandry Department, University of Missouri, the United States Department of Agriculture coöperating.

Numerous experiments have demonstrated that as the density of a population within a limited, closed system increases, the physiologic limitations imposed upon the individuals making up the population progressively increase. Thus, it has been shown that egg production of Drosophila confined within a limited space declines with increasing density of population.[1] Sea urchin sperm, inactive in the testis, have been shown to become very active at low concentrations in seminal plasma, and to exhibit a progressively increasing respiration rate accompanying increasing dilution with sea water.[2] Marine biologists are familiar with this phenomenon. A large increase in respiration rate of sperm of the fowl on reduction of concentration to 1/5 the original level has been reported.[3]

If the respiration of mammalian sperm cells similarly varies with concentration, serious errors could result when different samples are compared, since the sperm concentration of semen varies within wide limits. To test this point, we carried out, during the past year, measurements of the O_2 consumption rates of identical sperm, of either swine or sheep, suspended at various concentrations in identical media. The mean volume of boars' ejacula is around 200 cc with sperm concentrations commonly ranging from 2.5 million to over a billion per cc.[4] The mean volume of (Shropshire and Hampshire) rams' ejacula is 2 cc, with mean concentration in the neighborhood of 3 billion sperm per cc of semen during the months of greatest sexual activity.[5] These figures correspond closely to those for semen used in this study.

---

* Contribution from the Department of Animal Husbandry, Missouri Agricultural Experiment Station. Journal Series No. 778.

[1] Pearl, Raymond, *J. Exp. Zool.*, 1932, **63**, 57.

[2] Gray, J., *Brit. J. Exp. Biol.*, 1928, **5**, 337.

[3] Winberg, Hakan, *Arkiv. f. Zool.*, 1939, **32**A, No. 7.

Nine purebred Ramboullet rams 3 to 4 years of age were used, the total ejacula of 5 to 7 was combined to obtain conveniently large samples. The boars used were: one Poland China and 2 Chester White boars one year of age, and one Hampshire boar 2 years of age. The ejaculum of one boar was sufficient for each trial.

Semen samples were obtained with the artificial vagina[6] by student assistants. Sperm concentration was increased by centrifugation at gravity × 1400. Any possible injuries caused by centrifugation probably were consistent for all dilutions. Respiration was measured at 37°C, for one hour, with a modified Barcroft-Warburg respirometer.[†] [7] Sperm concentration was determined with a hemocytometer.

In the first series, boar semen with an initial concentration of one billion, or less, sperm cells per cc was diluted with seminal plasma. This apparently had no influence on respiration rates (broken lines in Fig. 1). Later, sperm were concentrated by centrifugation, and in some trials concentrations as great as 6 billion sperm per cc (upper limit of concentration of untreated ram sperm) were obtained.

In the second series, each semen sample was divided, by centrifugation and subsequent dilution with seminal plasma, into fractions

---

[4] McKenzie, Fred F., Miller, J. C., and Bauguess, L. C., *Mo. Agr. Exp. Sta. Res. Bul.* 279, 1938.

[5] McKenzie, Fred F., and Berliner, Victor, *Mo. Agr. Exp. Sta. Res. Bul.* 265, 1937.

[6] McKenzie, Fred F., *The Cattleman*, Sept., 1939.

[†] Accuracy of the apparatus was determined periodically by simultaneous respiration measurements of yeast suspension in each of the 6 manometer units. The error (mean deviation × 100/mean) was 2%. An O_2 consumption rate so high as to exceed the capacity of the respirometer ([7], p. 48) was never approached in these experiments.

[7] Dixon, M., *Manometric Methods*, Cambridge University Press, London, 1934

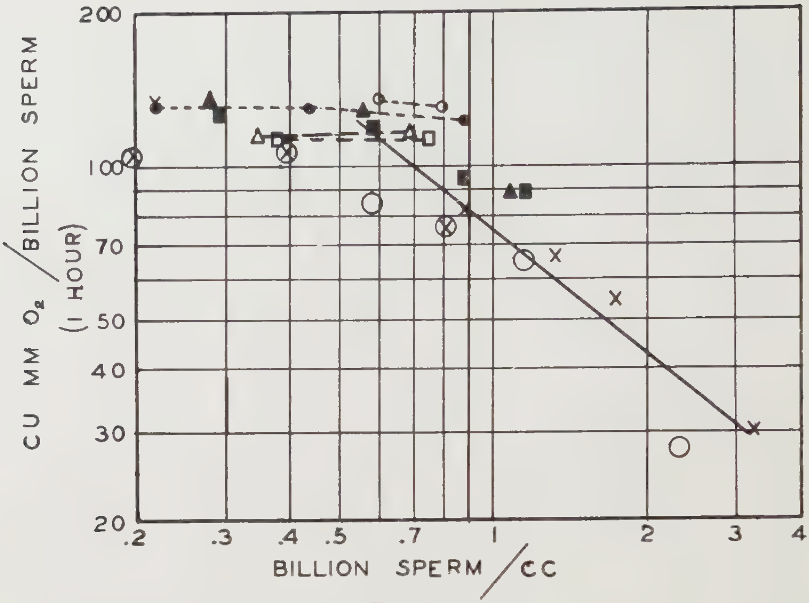


FIG. 1.

Oxygen consumption of boar sperm. (The symbols identify individual trials.)

which varied in concentration from 0.2 to 6 billion sperm per cc. While errors as large as 16% may have occurred in the initial determinations of sperm concentration,[8] the errors in relative concentrations of the sperm suspensions were merely the minute ones introduced in pipetting. Any effect of bacterial contamination was probably negligible.[9] In some trials the O_2 consumption of seminal plasma was determined[9] and subtracted from the seminal O_2 consumption to give that of the sperm. In the others, respiration of seminal plasma was estimated. In Fig. 1 are given the results of these trials with boar sperm, and in Fig. 2 with ram sperm. These figures show that, except at lower concentrations, a decrease in concentration was invariably accompanied by an increase in O_2 consumption of individual cells.‡

Any possible effect of pH as a variable was reduced, in the last series of trials, by the addition of phosphate buffer to the seminal plasma used in dilution, or suspension of washed sperm in phosphate buffer. The initial pH of all portions for any one trial was thus the same, and the final pH values were sometimes as close as 0.1 pH unit in the separate portions. The results are given in Figs. 3 and 4. The computed slopes of the various curves accompany the figures. Due to the lack of uniformity in slope, the respiration rate of a given sperm sample cannot be computed reliably at some theoretical concentration on the basis of data obtained at another concentration. *For comparison of respiration rates, therefore, semen samples must be diluted to a standard level of concentration.* The pH should also be controlled and seminal respiration rates corrected for O_2 consumption of seminal plasma.[9]

---

[8] Berkson, Joseph, Magath, Thomas B., and Hurn, Margaret, *Am. J. Physiol.*, 1940, **128**, 309.

[9] Winchester, C. F., and McKenzie, Fred F., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 455.

‡ The numerical data are plotted on logarithmic paper, the numbers shown being the original data while the relationships shown are those existing between the logarithms of these numbers. The use of this method in biological work has been discussed by Brody and Kibler.[10]

[10] Brody, S., and Kibler, H. H., *Mo. Agr. Exp. Sta. Res. Bul.* 328, 1941.

Why does seminal respiration decline with increasing sperm concentration? Gray[2] concluded that sperm cells exercise a mutual

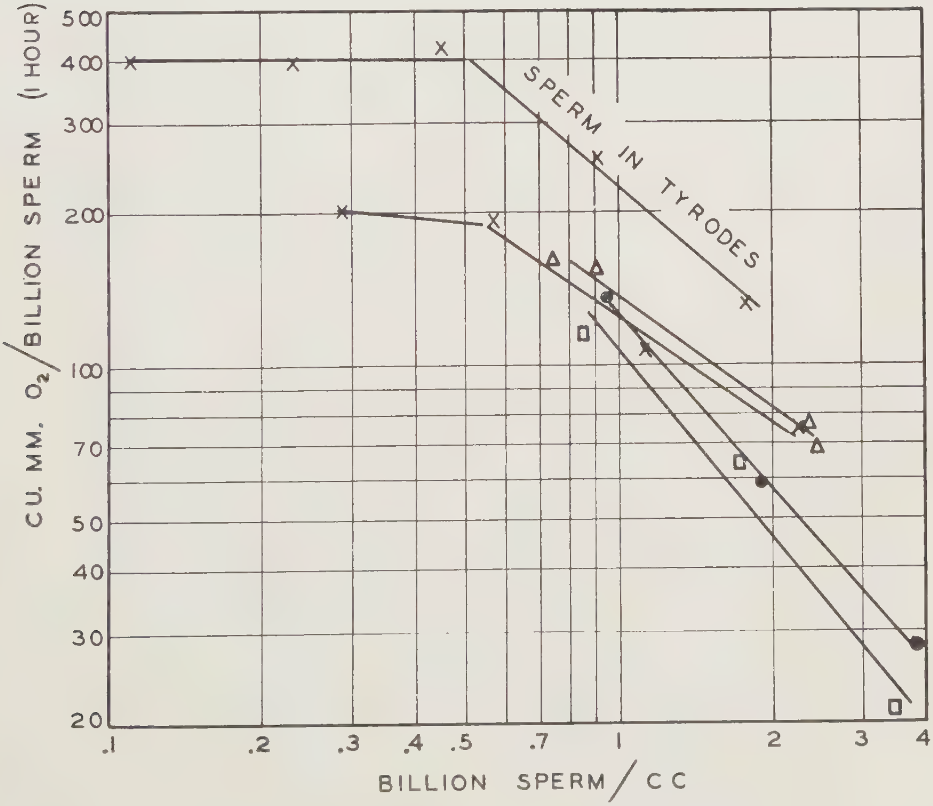


Fig. 2.
Oxygen consumption of ram sperm. (The symbols identify individual trials.)

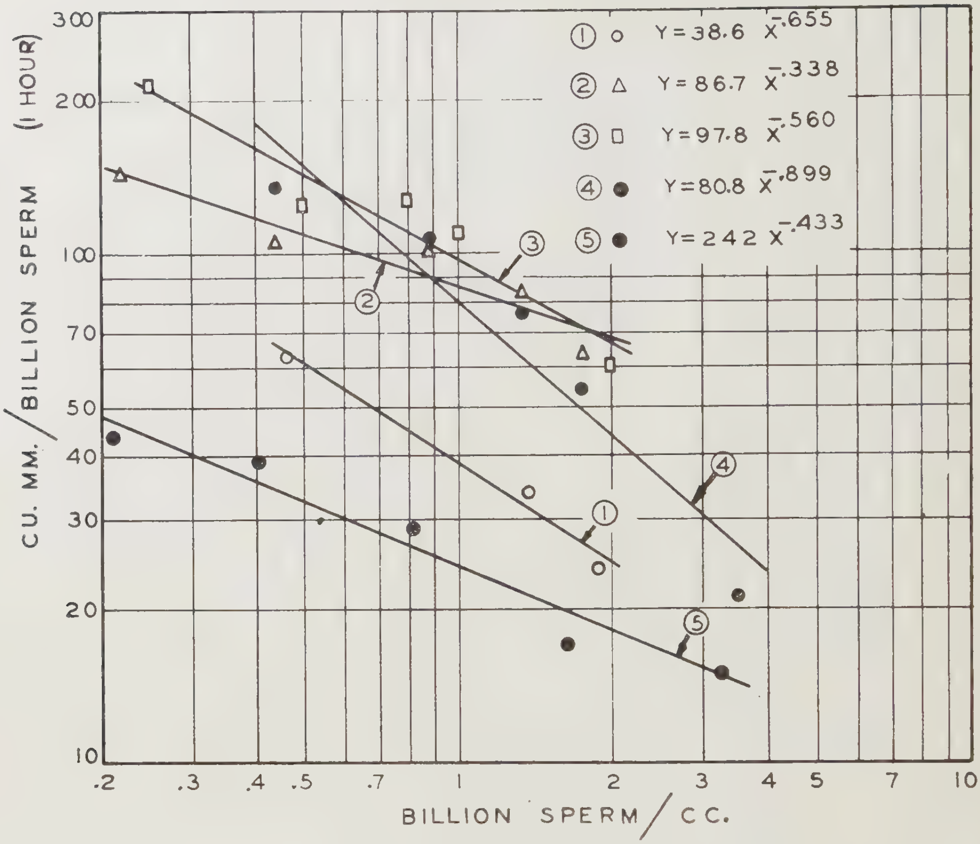


FIG. 3.
Oxygen consumption of boar sperm in buffered seminal plasma.

restraint, and showed that the effect probably was not due to reduction of O_2 available to individual cells since *more* O_2 sometimes was consumed by unit volume of suspension at relatively low concentration than at higher concentration. Our results confirm this observation. It may be significant that the respiration rate of leucocytes[11] is influenced by cell concentration in a manner analogous to that of sperm, while respiration rates of 2 non-motile organisms, yeast (Fig. 4) and bacteria,[12] are not. The fact that sperm in buffered media (Fig. 3 and 4) behaved similarly to those in seminal plasma (Figs. 1 and 2) appears to exclude differences in pH as a factor. The clumping together of cells cannot be regarded as the cause since this was observed as frequently at low as at high concentrations.

---

[11] Barron, E. S. G., and Harrop, G. A., Jr., *J. Biol. Chem.*, 1929, **84**, 89.

[12] Gerard, R. W., *Biol. Bul.*, 1931, **60**, 227.

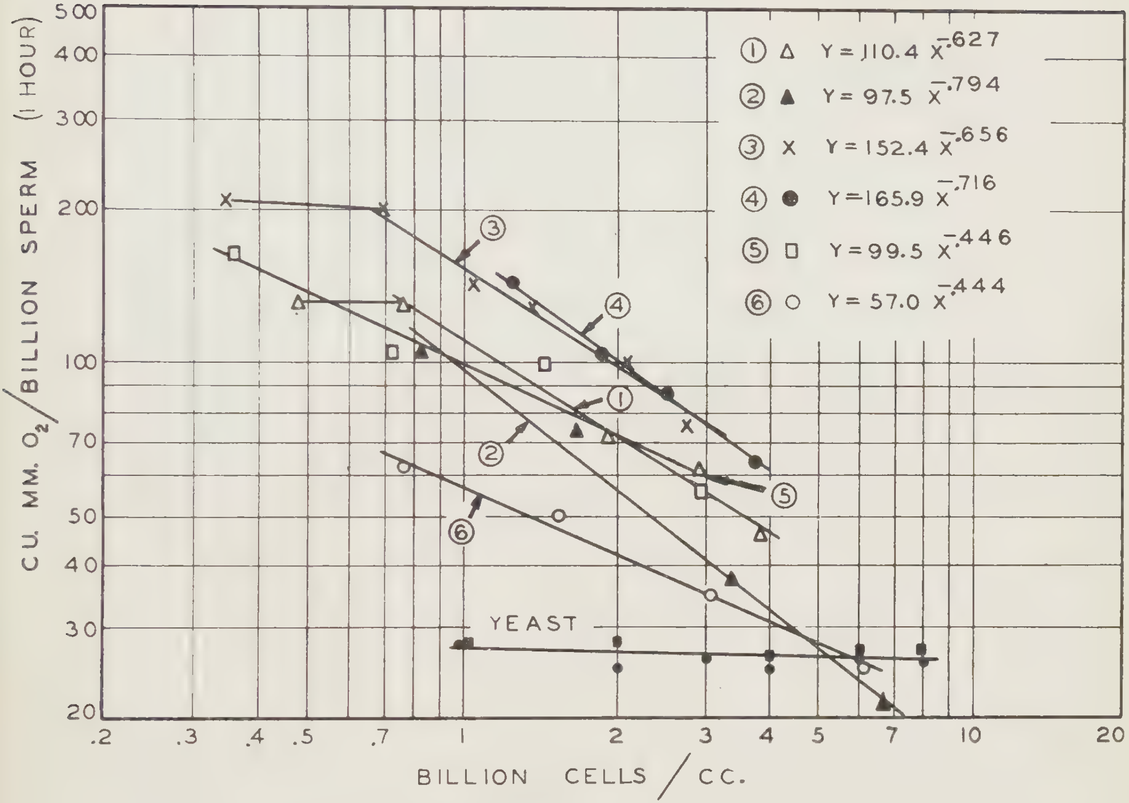


Fig. 4.
Oxygen consumption of ram sperm, and yeast cells, in buffered media.

Oxygen consumption curves of washed ram sperm in buffered solutions were about the same as those of normal semen; this is evidence that neither a seminal enzyme nor a metabolite is responsible.

Conclusions. 1. At sperm concentrations from 1 to 6 billion cells per cc an increase in concentration was accompanied by a decrease in sperm respiration rate. At concentrations of 0.2 to 1 billion sperm per cc this effect of concentration was not invariably observed. 2. In contrast with the behavior of sperm cells, respiration rates of yeast cells remained constant at concentrations from 1 to 8 billion cells per cc. 3. The effect of sperm concentration on sperm respiration rate does not appear to be due to changes in pH, diminished supply of O_2 to individual cells, an enzyme, or products of metabolism. 4. When comparisons are to be made of relative respiration rates of semen samples, the samples must be adjusted to the same level of sperm concentration.

13417

Influence of Hydrogen Ion Concentration on Respiration Rate of Sperm.*

C. F. Winchester and Fred F. McKenzie.

From the Animal Husbandry Department, University of Missouri, the United States Department of Agriculture coöperating.

Various authors[1, 2] have suggested that the respiration rate of sperm is influenced by the pH of the medium in which they are suspended, yet experimental evidence for this is lacking.† The optimum pH for sperm motility has been reported to be about 7.0 to 7.3[3] Motility is lost reversibly at about pH 4[3] and, despite contrary reports,† motility and respiration rate might be altered similarly by pH.

The problem is of interest from the point of view of cell metabolism as well as from that of the physiology of reproduction. We have attempted to answer the following questions: (a) Is there an optimum pH for sperm respiration? (b) Are there differences in the influence of pH on respiration of sperm of the various species? (c) Will small pH differences in buffered samples result in significant errors when comparisons are made of respiration rates?

All semen samples were obtained with the artificial vagina[4] by student assistants. The boars used were purebred and were of the following breeds and ages: one Poland China and 2 Chester White boars one year of age, and one Hampshire boar 2 years of age. The relatively concentrated early ejaculum of one boar was used in each trial. Nine purebred Rambouillet rams 3 to 4 years of age were used. Semen samples usually contained ejacula of 5 to 7 rams, and did not represent equal volumes from individuals.

Samples were adjusted to various pH levels by the addition to a given volume of semen of an equal volume of M/5 solution of phos-

---

* Contribution from the Department of Animal Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 770.

[1] Walton, Arthur, and Edwards, Joseph, *Am. Soc. An. Prod.*, 31st Annual Meet., 1938, 254.

[2] Sergin, N. P., *Dokl. Akad. seljskohoz. Nauk.* (U. S. S. R.), No. 2/3, 1939, 60; *An. Brd. Abst.*, 1939, **7**, 181.

† Oxygen consumption rates of sperm of mammalia were measured at various pH levels by Windstosser, K., *Klin. Woch.*, 1935, **14**, 193. The results are contradictory. Windstosser concluded that no definite correlation exists between respiration rate, motility, or pH of sperm.

[3] Allen, Edgar, *Sex and Internal Secretions*, Williams and Wilkins, Baltimore, Md., 1932.

[4] McKenzie, Fred F., *The Cattleman*, September, 1939.

phate buffer (NaH_2PO_4, Na_2HPO_4, or Na_3PO_4). The pH of each mixed sample was determined at the beginning and end of each trial with a Beckman pH meter standardized daily with buffers at pH 4 or 7. Respiration rates were determined at 37°C with a modified Barcroft-Warburg respirometer.[‡] [5] In each trial comparison was made between O_2 consumption rates of portions of a given sample which differed only in pH. The pH of the portion which exhibited the highest respiration rate is considered optimum, and respiration rates of the other portions are expressed as percentages of the highest rate. In boar samples, pH either did not change during the trials or increased up to 0.2 pH unit. The changes in pH of ram semen mixtures were towards the acid side and averaged less than those in boar semen. When the pH level changed during a trial, a mean value of the levels at the beginning and end of the trial was used in the calculations.

Results are given in Fig. 1. Each point represents a mean of 5 to 16 observations. The vertical lines from the points represent standard errors of the means.[6] Highest respiration rates were exhibited

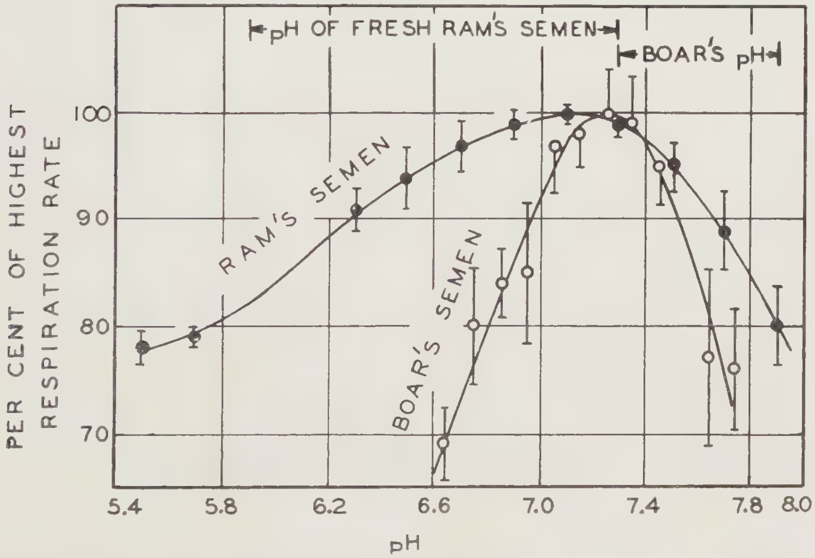


FIG. 1.

Influence of pH on respiration rate of sperm. Vertical lines from data points are standard errors of means. The pH values of fresh semen are those of McKenzie and coworkers.

[‡] Accuracy of the apparatus was determined periodically by simultaneous respiration measurements of yeast suspension in each of the 6 manometer units. The error (mean deviation × 100/mean) was 2%.

[5] Dixon, M., *Manometric Methods*, Cambridge Univ. Press, London, 1934.

by boar semen at pH 7.2 to 7.3, by ram semen at pH 7.0 to 7.2; these may be considered optima for these species. At pH levels beyond the limits of those given in Fig. 1 the respiration rates tended to approach that of seminal plasma.[7]

The data given for boar semen consist of results obtained separately with ejacula of 4 boars. They were combined after comparisons had shown that differences in respiration rates of samples from a given animal were as great as those of samples from different animals. The pH ranges of fresh semen of the boar and the ram, given in Fig. 1, are those reported by McKenzie and coworkers.[8, 9]

Conclusions. (1) Hydrogen ion concentrations of the media in which sperm of the boar and ram are suspended definitely influence respiration rates of the sperm. (2) Optimum pH for respiration of boar semen is 7.2 to 7.3, and for ram semen 7.0 to 7.2. (3) As the pH is raised or lowered from the optimum the respiration rate progressively declines. (4) Unit change in pH has significantly less influence on respiration rate of ram sperm than on that of boar sperm. (5) Differences of 0.4 pH unit or more are accompanied by significant differences in respiration rates of boar and ram semen. Conditions for valid comparisons of different samples are presented.

13418

Retinal Pigment Distribution in Relation to a Diurnal Rhythm in the Compound Eye of Dytiscus.*

THEODORE LOUIS JAHN AND VERNER JOHN WULFF.
From the Zoological Laboratories, State University of Iowa.

In the higher Malacostracans one manifestation of a persistent diurnal rhythm is the periodic migration of retinal pigment. Therefore, the discovery of a diurnal rhythm in the electrical response from

---

[6] Snedecor, George W., *Statistical Methods*, p. 54, Iowa State College Press, Ames, 1940.

[7] Winchester, C. F., and McKenzie, Fred F., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 455.

[8] McKenzie, Fred F., Miller, J. C., and Bauguess, L. C., *Mo. Agr. Exp. Sta. Res. Bul.* 279, 1938.

[9] McKenzie, Fred F., and Berliner, Victor, *Mo. Agr. Exp. Sta. Res. Bul.* 265, 1937.

* Aided by a grant from the Rockefeller Foundation for Work in Cellular Physiology.

the eyes of certain beetles (Jahn and Crescitelli[1]) led to the idea that retinal pigment migration might in some manner be concerned with the phenomenon.

More recently it has been demonstrated that the diurnal variation in the compound eyes of the beetle Dytiscus results in two distinct physiological states, called the night phase and the day phase, which are characterized by: (1) differences in wave form of the electrical response; (2) differences in the process of light adaptation; (3) differences in the type of high frequency oscillation obtained from the optic ganglia; (4) differences in the sensitivity threshold to light; and (5) differences in the response to flicker, (Jahn and Wulff[2, 3]). In order to interpret these phenomena, especially the last two, the possible influence of retinal pigment migration must be considered.

The purpose of this investigation was to determine the distribution of the retinal pigment in the compound eye of Dytiscus during the night and during the day and under conditions of extreme light and extreme dark adaptation.

Materials and Methods. Prior to use, specimens of *Dytiscus fasciventris* were kept in aquaria in the laboratory under conditions simulating those of the natural environment.

1. Preparation of dark-adapted animals.

Four animals were placed in an aquarium in a dark room for periods ranging from 15 to 36 hours to insure maximum dark-adaptation. Two animals were removed from this aquarium during the height of the day phase, about 10:00 a.m., and were dropped into a beaker of boiling water. After 10 minutes the beetles were removed from the hot water and decapitated, and their heads were immersed in Bouin's fixative for 24 hours. The material was then prepared for sectioning by a special technic, developed for hard materials by Prof. E. H. Slifer and Mr. T. Tahmisian of this laboratory.

The 2 remaining animals were removed from the aquarium during the height of the night phase, about 11:00 p.m., and treated in the manner described.

2. Preparation of light-adapted animals.

Animals for these experiments were taken from the general aquarium and mounted in a dark chamber so that only one eye was exposed to the stimulating light. The animals were maximally dark-adapted and then exposed to the 10,000 foot-candle light stimulus for 20 minutes. The beetles were then quickly removed from the chamber, killed and fixed as described. Animals were treated in this manner at the height of the day and night phases.

---

[1] Jahn, T. L., and Crescitelli, F., *Biol. Bul.*, 1940, **78**, 42.

[2] Jahn, T. L., and Wulff, V. J., submitted for publication, 1941.

[3] Jahn, T. L., and Wulff, V. J., submitted for publication, 1941.

Results. Comparisons were made of radial sections through the center of the eyes. The results are as follows:

A. The distribution of retinal pigment in the dark-adapted eye.

In the day-phase eye the distal pigment is concentrated markedly toward the corneal ends of the retinulae, as can be seen in Fig. 1, Plate I. This distribution of the distal pigment permits the light to stimulate the photosensory elements over a considerable angular range. The proximal pigment seems to be concentrated on the distal side of

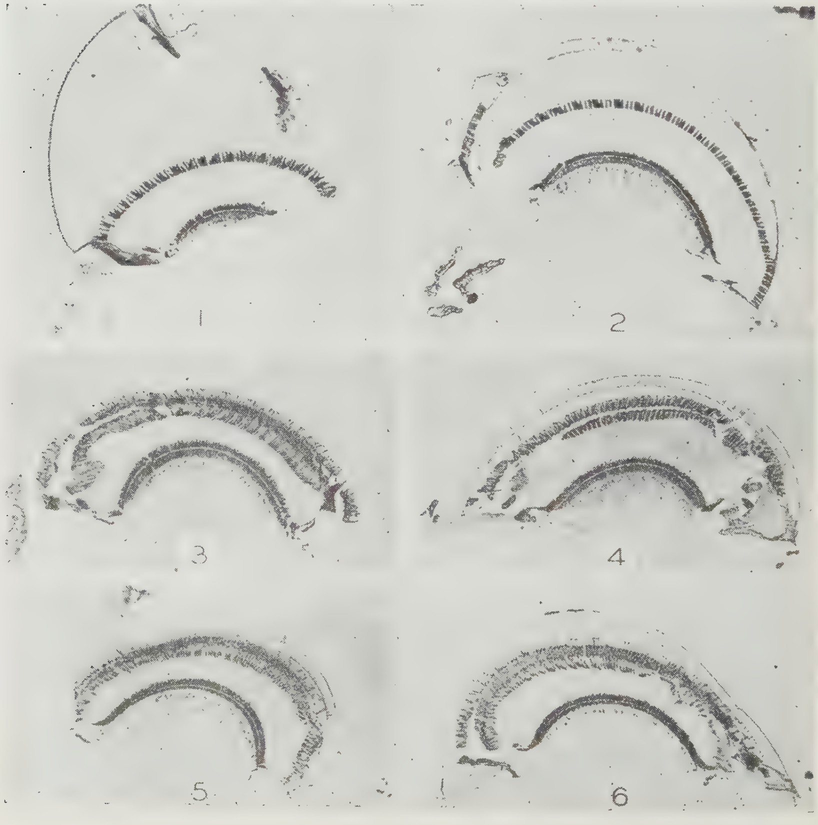


PLATE 1.

Radial sections through the compound eyes of the beetle, *Dytiscus fasciventris*. Fig. 1 and 2, dark-adapted day- and night-phase eyes, respectively. Fig. 3 and 4, right and left light-adapted day-phase eyes, respectively. Fig. 5 and 6, right and left light-adapted night-phase eyes, respectively. In the animals used for Fig. 3 to 5, only the night eye was illuminated. For full detail refer to text.

the basement membrane and dispersed on the proximal side, but the significance of this distribution is not known.

In the night phase the distribution of the pigment in the dark-adapted eye is precisely the same as that for the day phase (Fig. 2, Plate I).

B. Distribution of the pigment in the light-adapted eye.

In the light-adapted day-phase eye (Figs. 3 and 4, Plate I) the distal pigment is considerably more widely dispersed than in the dark-adapted eye, so that the distal half of the retinulae is sheathed with the dark pigment. This means that the light which stimulates the sensory elements must strike the ommatidia perpendicularly or nearly so. Most of that light which strikes at an angle will be absorbed by the distal pigment. The obvious result of this is to lower the subjective intensity of the stimulating light. The proximal pigment is concentrated on both sides of the basement membrane, and the meaning of this distribution is not obvious. The distribution of pigment is the same for both the right and left eye, although only one eye was stimulated by light. This indicates that the nervous or neuro-humoral systems are involved in pigment migration.

In the light-adapted night-phase eye (Figs. 5 and 6, Plate I) the distribution of the pigment is identical with that in the light-adapted day-phase eye. The distribution in both right and left eyes is the same, although only one eye was illuminated.

Discussion. It is quite apparent, in view of the facts just mentioned, that migration of retinal pigment is totally unrelated to the physiological diurnal rhythm present in the eyes of Dytiscus. Hence, the peculiarities of visual function, by which this diurnal rhythm is identified, must have their origin in the physiological mechanism responsible for visual phenomena. This conclusion is supported by the fact that in the crayfish, which has a diurnal cycle of pigment migration, there is apparently no diurnal change in the electroretinogram (Jahn and Crescitelli[1]).

Thus, the fact that the dark-adapted night-phase eye has an intensity threshold 1000 times lower than the dark-adapted day-phase eye (Jahn and Wulff[2]) attains considerable significance. At present the only reasonable explanation seems to be that the concentration of photosensitive materials is greater in the dark-adapted night-phase eye than in the dark-adapted day-phase eye. These sensitivity relationships are not true for the partially light-adapted eye, which has an intensity threshold about the same during the day and night phase, and the maximally light-adapted eye, where the sensitivity relations are totally reversed (Jahn and Wulff[2, 3]). This may then be interpreted as meaning that the process of light adaptation is more pronounced in the night-phase eye, resulting in a lower concentration of photosensitive materials than is present in the day-phase eye light-adapted to the same extent. Why this difference in the effect of light should exist must be left to future investigation. The differences in response to flicker, described elsewhere (Jahn and Wulff[3]), can be explained in a similar manner.

The usual migration of proximal and distal retinal pigment under the influence of light is sufficiently well known not to require further discussion (Parker[4]).

Conclusions. 1. The distribution of retinal pigment is similar in the dark-adapted day-phase and dark-adapted night-phase eyes. 2. The distribution of retinal pigment is similar in the light-adapted day-phase and light-adapted night-phase eyes, as well as in the illuminated and non-illuminated eyes of the same animal. 3. The diurnal variations in visual function, described elsewhere, are, therefore, independent of retinal pigment migration and originate in the physiological mechanism of the visual process.

13419

Influence of a Visual Diurnal Rhythm on Flicker Response Contours of Dytiscus.*

THEODORE LOUIS JAHN AND VERNER JOHN WULFF.
From the Zoological Laboratories, State University of Iowa.

A physiological diurnal rhythm is present in the compound eye of the predaceous diving beetle, *Dytiscus fasciventris*. This diurnal rhythm results in two distinct functional states of the eye which are referred to as the night phase and the day phase. These physiological states may be identified by several characteristics which are enumerated elsewhere (Jahn and Wulff[1, 2]).

The measurement of subjective flicker fusion frequency at various intensities of stimulating light has proven to be an excellent index of the physiological state of photoreceptors. The purpose of this

---

[4] Parker, G. H., *Ergebn. der Biol.*, 1932, **9**, 239.

* Aided by a grant from the Rockefeller Foundation for Work in Cellular Physiology.

[1] Jahn, T. L., and Wulff, V. J., submitted for publication, 1941.

[2] Jahn, T. L., and Wulff, V. J., submitted for publication, 1941.

investigation is to measure the flicker fusion frequencies of the Dytiscus compound eye during the night phase and day phase by means of the objective method of determining from the electroretinogram when fusion of the individual electrical responses occurs.

Materials and Methods. Prior to use as experimental material specimens of *Dytiscus fasciventris* were kept in the laboratory in aquaria under conditions simulating those of the natural environment.

The method of recording the potential from the eye was similar to that described previously (Jahn and Crescitelli[3]). The electrical leads were made from the corneal surface of the eyes of the beetle mounted in the dark chamber. One eye only was exposed to the stimulating light. The recording device consisted of a condenser-coupled high-gain amplifier, a cathode ray oscillograph and a camera. Just previous to experimentation the animals were maximally light-adapted, and progressive dark adaptation occurred during the course of the experiment as the intensity of the stimulating light was decreased. The flicker consisted of a 1 to 1 ratio of light and darkness of variable frequency, which could be adjusted in steps of 4 cycles per second. The maximum intensity of light was 10,000 foot-candles.

Results. A. Measurement of flicker fusion frequency at high intensity. The results of the high intensity (10,000 foot-candles) flicker response experiments during the day and night phases are shown in Fig. 1. As determined in this manner the ability of the day-phase eye to distinguish flicker (as indicated by the ERG) is not lost until the flicker frequency is increased to almost 60 stimuli per second.

In the light-adapted night-phase eye fusion was observed at 40 stimuli per second, a frequency which is significantly lower than the fusion frequency of the same eye during the day phase. Comparison of the magnitude of the response of the day- and night-phase eyes to the same flicker frequencies, records B and D, and records C and E, tend to emphasize this difference.

B. Flicker response contours for the day- and night-phase eyes. In these experiments each of the 3 beetles used was subjected to only 2 investigations; one during the day phase and one during the night phase. In each experiment the flicker fusion was determined over an intensity range beginning with 10,000 foot-candles and decreasing gradually to threshold value. The intensity was controlled with Wratten neutral filters.

---

[3] Jahn, T. L., and Crescitelli, F., *J. Cell. and Comp. Physiology*, 1938, **12**, 39.

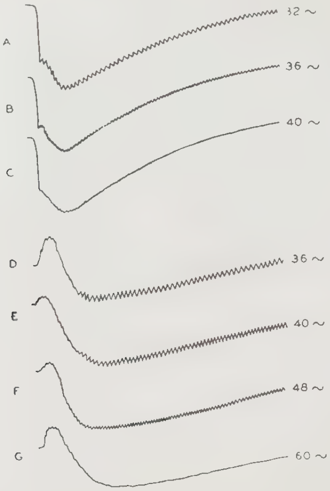


FIG. 1.

Flicker responses of the night- and day-phase eyes. Records A through C are from the night-phase eye; records D through G from the day-phase eye of the same animal. The intensity of the stimulating light was 10,000 foot-candles for all records. The amplifier was operated at a constant intermediate gain and a 2.9 second time-constant. Refer to text for further details.

The responses of the eye to flicker were recorded on constantly moving sensitized paper. At each intensity records were taken of frequencies immediately above and below the critical fusion frequency. When the records indicated a smooth base line (compared to the control) it was assumed that the eye was not able to distinguish the flicker. The curves of Fig. 2 demonstrate the relationship between flicker fusion frequency (in cycles per second) and the logarithm (base 10) of the light intensity. Each point on the graph represents the average value from three experiments on 3 beetles.

These curves illustrate that the flicker response of the day- and night-phase eyes differ over most of the intensity range. At the high intensity end the flicker fusion of the light-adapted night-phase eye is considerably lower than that for the light-adapted day-phase eye. As one approaches the low intensity portion of the graph the two curves approach and finally cross each other, indicating that the flicker fusion at low intensity is higher for the now partially dark-adapted night-phase eye than for the partially dark-adapted day-phase eye.

Discussion. From experiments published elsewhere (Jahn and Wulff[1]) it is known that, in the maximum dark-adapted condition, the night-phase eye is approximately 1000 times more sensitive to light than is the day-phase eye (threshold measurements). This difference in sensitivity is entirely unrelated to any differential pig-

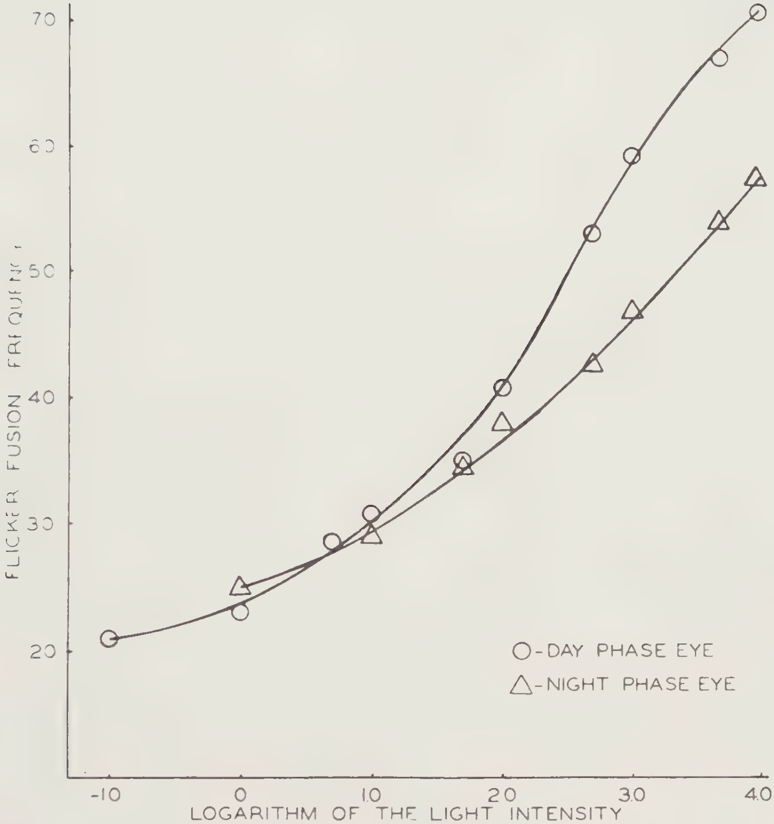


Fig. 2.

Flicker fusion response contours of the day- and night-phase eyes. The frequency (in cycles per second) at which fusion occurs is plotted against the logarithm (to the base 10) of the light intensity. The amplifier was operated at the highest gain which would permit a smooth base line and at a time constant of 0.1 second. Refer to text for further details.

ment distribution (Jahn and Wulff[2]) for the retinal pigment has precisely the same distribution in both the dark-adapted day- and night-phase eyes. Hence, this difference in sensitivity indicates a basic difference in the functional states of the eyes.

Although the results of the two types of experiments reported in this paper are not directly comparable, they both demonstrate the fact that at high intensities the flicker fusion frequency for the day-phase eye is greater than for the night-phase eye. On the basis of the established fact that flicker fusion frequency is a measure of the subjective sensitivity of the eye to light (Ferry-Porter law), this observation means that the intensity perception of the light-adapted day-phase eye is considerably greater than that of the light-adapted night-phase eye. In other words, the light-adapted day-phase eye is more sensitive to light than is the light-adapted night-phase eye. Since the retinal pigment distribution of both of the light-adapted eyes is identical, the basis for this difference in sensitivity must lie in the physiological mechanism of the eye.

This profound reversal of sensitivity relations of the night- and day-phase eyes from the dark-adapted state to the light-adapted state can not be satisfactorily explained at present. It is certain, however, that the reversal is brought about by light and that light adaptation is much more pronounced in the night-phase eye than in the day-phase eye.

Since the dark-adapted night-phase eye is considerably more sensitive to light than is the dark-adapted day-phase eye one would expect that, as the dark-adapted state is approached, the flicker fusion frequency of the night-phase eye would gradually become greater than that of the day-phase eye. The curves of Fig. 2 corroborate this expectation. At high intensity, where the eyes are maximally light adapted, the spread between the curves is large. As the intensity is progressively decreased the eyes become more dark-adapted, and the sensitivity of the night-phase eye increases more rapidly than that of the day-phase eye with the result that the curves approach and finally cross each other. Beyond the crossing point the curves again separate, the night-phase curve now higher than the day-phase curve. The threshold sensitivity of the eyes prevented carrying the experiment to lower intensities.

In general, these flicker experiments indicate that the day-phase eye and the night-phase eye of the beetle, *Dytiscus fasciventris,* represent physiological states which are so different that they may be considered functionally as two distinct photoreceptors.

The objective method of measuring flicker fusion frequency is quite different from the subjective methods ordinarily used (Hecht,[4] and Crozier[5]). At present no data are available which can demonstrate the exact relationship between these methods, but it is assumed that the flicker contour curves obtained by the two methods follow parallel courses. This assumption is based on various demonstrations (Granit,[6] and Therman[7]) that the magnitude of certain components of the electroretinogram are closely related to certain visual functions.

---

[4] Hecht, Sebig, *Phys. Rev.*, 1937, **17**, 239, and earlier publications.

[5] Crozier, W. J., *J. Gen. Physiol.*, 1941, **25**, 75, and earlier publications.

[6] Granit, R., *Documenta Opthalmologica*, 1938, **1**, 7.

[7] Therman, P. L., *Acta Societatis Scientiarum Fennicæ*, New Series B, 1938, **2**, No. 1.

Conclusions. 1. The flicker fusion frequency-intensity relations differ for the light-adapted day- and night-phase eyes. 2. This difference is marked in the high intensity range and decreases as the threshold intensities are approached. 3. In view of data published elsewhere the reversal of sensitivity relationships indicates that the process of light-adaptation is much more pronounced in the night-phase than in the day-phase eye. 4. The flicker response contour and other relationships confirm the conclusion, made elsewhere, that the diurnal rhythm present in the eyes of these beetles results in two physiological states which are so different that they may be considered functionally as separate and distinct photoreceptors.

13420

Sexual Behavior of Intersexual Domestic Fowl.*

L. V. DOMM AND DAVID E. DAVIS.

From the Whitman Laboratory of Experimental Zoology, The University of Chicago.

This investigation concerns the behavior of intersexual birds, produced by the injection of estrogens into the egg on or before the fourth day of incubation. The males, as a result of this treatment, develop into intersexes of varying degrees of femininity, as indicated by behavior patterns and plumage.[1] The females are essentially normal except for the occasional occurrence of anomalous oviducts and the greater frequency of right oviducts.[2] The use of intersexual individuals permits the beginning of an analysis of the various components of sexual behavior, which, in this instance, is attempted from a psycho-analytical viewpoint.

The birds were kept in pens measuring 8′ x 17′ and containing from 5 to 9 birds of both sexes. The males were arbitrarily classified on the basis of plumage characters into 4 intergrading classes

---

* This investigation was aided by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of The University of Chicago. Grateful acknowledgment is made to Dr. Erwin Schwenk of the Schering Corporation for the Progynon-B, Dr. Oliver Kamm of Parke, Davis & Company and Dr. Edward A. Doisy of the St. Louis University School of Medicine for the Theelin, and to Dr. J. A. Morrell of Squibb and Sons for the Stilbestrol and Amniotin used in these experiments.

[1] Domm, L. V., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 310; Domm, L. V., *Anat. Rec.*, 1940, **78** (suppl.), 144.

[2] Domm, L. V., *Anat. Rec.*, 1940, **78** (suppl.), 145.

as follows: I. Males which are essentially masculine in general appearance. II. Males which show a prominent scattering of female feathers on hackle, back, and saddle, and a lesser number of scattered female feathers on the breast. III. Males which have a female or nearly female hackle, back, and saddle, while the breast and tail, though predominantly female, still show many scattered male feathers. IV. Males which are practically indistinguishable from the normal female in general appearance.

The experimental technic consisted of introducing the individual to a rooster, to a hen, or to a stuffed dummy, mounted in the squatting copulatory pose. Several procedures were used to isolate the intersexual bird from other birds in order to increase its sexual drive.

The peck order or rank of social dominance was observed in each pen. In general, the most masculine birds were at the top and the most feminine at the bottom of the ranking among the males.

A brief description of the sexual behavior of normal domestic fowl is a prerequisite for an understanding of the behavior of intersexual birds. The sex of a newcomer is determined by behavior. The rooster "waltzes", *i. e.,* lowers one wing and kicks the leg on the side of the lowered wing, thereby inducing the newcomer to either squat or raise the neck hackle feathers. When a bird is introduced to a rooster, he at once pursues, seeking to determine its sex. If it is a receptive female, she squats for copulation. If, on the other hand, it is a male, it raises the neck hackle and thereupon begins a fight which settles its social rank in the group. This fighting, which is initiated by the raising of the hackle, is the alternative to copulation. Two females fight in a similar but less violent manner. The rooster copulates with the stuffed dummy in a behavioristically perfect manner.

The sexual actions of intersexual males showed gradations from essentially perfect masculine behavior patterns to definitely neutral or inactive behavior. A progressively more complicated and cumulative series of behavior patterns coincided with the degree of masculinity of the plumage and of the behavior as a whole. Several stages in behavior preliminary to copulation may be distinguished: (1) The lowered wing. The bird lowers the wing to the ground and bends the head. The action then is ended and the bird may walk away. (2) Waltz and stop. The bird lowers one wing, kicks with one foot, then abruptly rears up and stops. (3) Waltz and circle. The bird lowers one wing, kicks, and then circles around the female. (4) Waltz and grab. The bird waltzes in a perfect manner and grabs the female by the neck hackle or comb. This last pattern is sometimes followed by: (5) The complete copulatory behavior. In general, birds of Class 4 seldom went beyond Stage 1. Birds of Class 3 seldom went beyond Stage 3. Birds of Class 2 commonly performed Action 4. Birds of Class 1 copulated occasionally. Fertile copulations were found only in some Class 1 individuals. The behavior of the intersexual birds with the dummy showed identical patterns but in general was on a lower level of activity, probably due to the lack of motion on the part of the dummy.

Summary. The behavior of intersexual male domestic fowl shows gradations from essentially normal masculine copulatory patterns to neutral or inactive behavior, coinciding in general with the degree of masculinity of plumage.

13421

The Sexual Behavior of Hormonally Treated Domestic Fowl.*

DAVID E. DAVIS AND L. V. DOMM.

From the Whitman Laboratory of Experimental Zoology, The University of Chicago.

The stereotypy of avian behavior patterns makes birds excellent experimental material for a study of the factors influencing behavior. Some of these factors can be segregated by the injection of appropriate hormones into normal and castrate individuals.

In order to analyze the relation of certain endocrines to sexual behavior patterns, a series of experiments was undertaken on domestic fowl of the Brown Leghorn variety. Normal roosters, capons, and sinistrally and bilaterally ovariectomized poulards were used in our experiments. In the present series, capons and bilaterally ovariectomized poulards were injected daily with testosterone propionate and estradiol (progynon-B), sinistrally ovariectomized poulards were injected with testosterone, and normal roosters received stilbestrol. The behavior of these individuals was tested, generally on alternate days, in the following manner: Each experimental bird, alone in his own pen, was introduced at suitable intervals to a normal female, to a normal rooster, or to a stuffed female which was mounted in the squatting, copulatory position.

---

* This investigation was aided by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of The University of Chicago. Grateful acknowledgment is made to Dr. Erwin Schwenk of the Schering Corporation for the Testosterone Propionate and Progynon-B, and to Dr. J. A. Morrell of Squibb and Sons for the Stilbestrol used in these experiments.

Two capons receiving 2.50 and 3.75 mg testosterone daily, copulated, crowed, and waltzed within 2 weeks. A third very nervous capon, receiving 1.25 mg, never copulated. Similarly, 3 capons receiving .50, 1.00, and 1.50 mg estradiol daily, copulated in 18 days. Another series receiving 1.00, 1.50, and 2.00 mg estradiol copulated within a week. Two capons receiving 1.50 and 2.00 mg stilbestrol copulated. A third very nervous bird, receiving 1.00 mg, never copulated. However, in contrast with the effects of testosterone, the estrogen-injected birds were not known to crow, and rarely "tidbitted"[†] or "waltzed". Furthermore, the vigor of these estradiol-injected birds, as revealed in copulation and general activity, was greatly reduced. The "tidbit" is considered a substitute activity frequently observed when the rooster is frustrated. Two normal roosters, receiving 2.5 and 5.0 mg stilbestrol, stopped crowing by the tenth day but continued to copulate, although in a listless manner, throughout the 46 days of the experiment. One of them waltzed and tidbitted listlessly a few times during the last week. The other bird was not observed to waltz or tidbit after 10 days of injection. Postmortem revealed tremendously reduced testes whose weight per bird was 75 and 110 g. Stilbestrol and estradiol both brought about normal feminization of growing feathers. The estrogenized capons show hypertrophy of the cloaca approximating that of the laying hen.

Three bilaterally and three sinistrally ovariectomized poulards receiving 1.25, 2.50, and 3.75 mg testosterone, crowed and waltzed within 21 days. However, these poulards never copulated with a normal female or the dummy during the 89 days of injections. Instead they violently pecked the normal female and the dummy. Similarly, the sinistrally ovariectomized poulards, known to possess left ovotestis, became more aggressive following injections of testosterone propionate. They showed a greater frequency of crowing and waltzing but never copulated. Two bilaterally ovariectomized poulards receiving .50 and 1.00 mg estradiol became lethargic and ignored the dummy and other females in all tests. One individual on the 18th day of injection squatted for the normal rooster and received copulation. A second bilaterally ovariectomized individual reacted similarly on the 42nd day, receiving the male in a normal manner. The third bird receiving 1.50 mg did not squat.

Summary. Both androgen and estrogen, injected into capons, induced the masculine copulatory behavior but only androgen induced crowing and increased or induced the "tidbitting" and "waltzing". In bilaterally ovariectomized poulards, androgen induced crowing and waltzing but did not induce the masculine copulatory behavior while estrogen, on the contrary, induced the feminine squatting behavior. These results would seem to suggest that certain behavior patterns are common to both sexes of the fowl and may be induced by the appropriate hormone. The masculine copulatory behavior can be induced by both androgen and estrogen but only in genetically determined males.

[†] "Tidbit" is used as a shorthand term for calling the hen to share a real or imaginary morsel.

13422 P

Toxicity of Intravenous Paraldehyde.

C. L. Burstein and E. A. Rovenstine.

From the Division of Surgery, Department of Anesthesia, New York University College of Medicine, New York City.

Intravenous paraldehyde for anesthesia of short duration has been advocated frequently by clinicians.[1-4] The rapid induction and quick recovery following its use have been factors in formulating the impression that it is without toxic effects. The numerous clinical reports contrast sharply with the paucity of experimental investigations, and this stimulated a study to determine the safety of intravenous paraldehyde administration in experimental animals. In accordance with the regularly recommended clinical practice the drug was injected intravenously undiluted, at the rate of 0.5 cc per second.

Thirty cats, 20 dogs and 20 rabbits were used.

The Minimum Anesthetic Dose (smallest dose that produced anesthesia) was found to be 0.3 cc per kilo body weight in all 3 species. The Minimum Lethal Dose (dose at which 50% of the animals died) was found to be 0.45 cc per kilo (cats, rabbits) and 0.50 cc per kilo (dogs). These figures demonstrate a margin of safety of low order.

Autopsy findings in the animals given lethal doses were similar.

---

[1] Noel, H. L. C., and Souttar, H. S., *Lancet*, 1912, **2**, 818.

[2] Beauchemin, J. A., Springer, R. G., and Elliott, G. A., *M. Times and Long Island M. J.*, 1935, **63**, 179.

[3] Miller, A. H., *Anesth. and Analg.*, 1936, **15**, 14.

[4] Robinson, L. J., *New Eng. J. Med.*, 1938, **219**, 114.

Those expiring within 10 minutes following intravenous paraldehyde had massive diffuse pulmonary hemorrhage and dilatation of the right heart. When death occurred 6 to 24 hours after anesthesia, acute pulmonary edema was evident. Animals recovered from anesthetic doses looked poorly. After one week autopsy revealed multiple pulmonary hemorrhages. Histo-pathological changes in other viscera were also observed. A further investigation of these is in progress.

Other observations during this study were directed toward respiratory and circulatory reactions.

In accord with clinical reports the respiratory rate was increased. Immediately after intravenous injection of paraldehyde there was often an apnea for several seconds. This was followed by rapid, shallow respirations; the rate increasing from 16-20 to as much as 120 per minute. Coughing was observed frequently and cyanosis was noted which became progressively more intense.

With doses producing anesthesia there was a prompt decrease in arterial blood pressure and an accelerated pulse rate. Even in animals which survived 12 to 24 hours, there was an immediate fall in the arterial blood pressure of more than 50%.

Conclusion. The intravenous administration of paraldehyde as recommended for clinical anesthesia is not without danger. In experimental animals there is a narrow margin of safety (Minimum Anesthetic Dose-Minimum Lethal Dose). Massive diffuse pulmonary hemorrhages and dilatation of the right heart occur when animals are killed with minimum lethal doses. Pulmonary hemorrhages are present in animals having recovered from anesthetic doses.

13423 P

Metabolism in Perfused Dog's Head During Sodium Pentobarbital Depression ann Metrazol* Stimulation.

C. A. HANDLEY, H. MORROW SWEENEY AND BRUCE T. BROOKMAN.

From the Department of Physiology and Pharmacology, University of South Dakota School of Medicine, Vermillion, So. Dak.

Previous studies on the effects of central nervous system depressants on brain metabolism have been largely confined to *in vitro* measurements of the oxygen consumption of minced brain tissue or slices (Quastel[1]). Determinations of the arterio-venous oxygen and glucose differences of cerebral blood in intact dogs after sodium pentobarbital administration indicated that the uptake of both were significantly reduced during depression. When pentamethylenetetrazol (metrazol) was then administered to the depressed dogs the oxygen and glucose uptakes by the brain were increased or restored to normal.[2] These measurements were possibly complicated by changes in blood flow and thus did not actually indicate the metabolism. Perfused dog head experiments suggested a method for making true metabolism studies.

---

* Metrazol was supplied by the Bilhuber-Knoll Corporation.

The perfusions were made with a Dale-Schuster double pump.[3] One unit of the pump was used to perfuse isolated lungs for oxygenating the blood. The head was perfused with the other unit by cannulating the common carotid artery and external jugular on one side, starting the pump and then cannulating the vessels on the other side. By using this technic, the blood flow to the head was not interrupted at any time. Venous blood samples were obtained from the previously exposed superior sagittal sinus. Arterial samples were taken from a common carotid artery. Oxygen content of the samples was determined according to the method of Van Slyke and Neill,[4] glucose by the method of Hagedorn and Jensen.[5] After control blood samples had been taken, sodium pentobarbital was administered. Five minutes later samples were again taken. Metrazol was then administered and the third set of samples were taken after

TABLE I.
Glucose and Oxygen Uptake by the Brain in Perfusion Experiments.
Uptake in cc of oxygen and mg of glucose per minute.

Exp.		Control	Pentobarbital	Metrazol	Pentobarbital
1	Oxygen	24.7	16.9	19.3	14.8
	Glucose	16	9	15	11
2	Oxygen	15	9.5	13.2	—
	Glucose	18	11	20	—
3	Oxygen	17.3	12.3	27	16
	Glucose	21	6	30	7
4	Oxygen	24.7	18.4	20.4	—
	Glucose	39	22	36	—
5	Oxygen	13.5	7.3	19.2	14.5
	Glucose	9	6	16	2

---

[1] Quastel, J. H., *Physiol. Rev.*, 1939, **19**, 135.

[2] Handley, C. A., and Sweeney, H. Morrow, *Am. J. Physiol.*, 1941, **133**, 314.

[3] Brookman, Bruce T., and Sweeney, H. Morrow, *Am. J. Physiol.*, 1941, **133**, 226.

[4] Van Slyke and Neill, *J. Biol. Chem.*, 1924, **61**, 523.

[5] Hagedorn and Jensen, *Biol. Chem. Z.*, 1923, **135**, 46.

a similar interval. The amounts of the drugs added to the perfusion blood were of the order of 10^{-4} molal. The blood flow was determined at the time the samples were collected by measuring the return flow in a graduated cylinder.

Table I, representing 5 perfusion experiments, shows that following sodium pentobarbital both the glucose and oxygen uptake by the brain was reduced. Following metrazol administration these figures were again increased to about the control levels. It will be noted that the uptake values after metrazol were appreciably greater in 2 of the experiments (3 and 5) than the control values. The probable explanation is that the dogs were given sodium pentobarbital in preparing them for the perfusion and although blood from unnarcotized animals was used for the perfusion, enough narcotic was present in the perfused head to produce some depression at the beginning of the experiment. In some of the experiments, a second injection of the barbiturate was given after the metrazol. The uptake values were again reduced by this procedure. The results of these experiments indicate that barbiturate narcosis inhibits metabolism in the brain, while metrazol has a tendency to reverse this effect.

The corneal reflex served as a guide to the degree of depression in the perfused head. It was present at the beginning of the perfusion, depressed or absent after sodium pentobarbital administration and restored by metrazol.

Summary. The results of perfused head experiments show that during sodium pentobarbital depression there is a measurable reduction in both oxygen and glucose utilization. Metrazol administered during this depression returns the utilization of both to about the control levels or beyond.

13424

A Chemotherapeutic Agent with Osteotropic Properties.

N. ERCOLI. (Introduced by E. Chargaff.)

From the Department of Pharmacology, University of Padua, Italy, and the Pharmacology Laboratory of Hoffmann-La Roche, Inc., Nutley, N.J.

In a search for chemotherapeutic agents with specific osteotropic properties, a study was made of the general mechanism of drug fixation in bone. It has been shown in previous studies[1] that the following properties are necessary for the fixation of drugs in the inorganic material of bone tissue: 1. Electronegativity of the substance. 2. High degree of diffusibility in the dissolved state. 3. Low solubility of its calcium salt. 4. Formation *in vitro* of an insoluble complex with calcium phosphate. (Properties described under 3 and 4 are interrelated, in that only anions giving slightly soluble calcium salts are adsorbed irreversibly by calcium phosphate.)

---

[1] Ercoli, N., *Kongressberichte des XVI Internationalen Physiologenkongresses,* Zürich (Switzerland), 1938.

For the purposes of this study, a colored bismuth compound (sodium salt of bismuth 1.2-dihydroxy-anthraquinone 3.sulfonic acid) was prepared which meets the above conditions, namely: 1. In this compound Bi is anionic, as demonstrated by electrolysis. 2. The compound is highly diffusible *in vivo* and *in vitro*. In 1.5% agar, the speed of diffusion is 16 mm in 16 hours as compared to that of Congo Red with 10 mm in 16 hours.

In mice, urinary elimination of the bismuth complex occurred 15-20 minutes after i.p. injection of 0.18 mg of bismuth per kg body weight. 3. The calcium salt of the compound has a low solubility. By adding $CaCl_2$ to a solution of the Bi preparation, the calcium salt is precipitated. 4. Ten grams of calcium phosphate adsorb the entire quantity present in 100 cc of a Mol/20 solution of the bismuth preparation. After filtering and washing the calcium phosphate with water, the adsorbed Bi compound can be detected quantitatively.

Sodium bismuth dihydroxy anthraquinone sulfonate, when injected into mice intraperitoneally in doses of 1.5 mg of bismuth per kg of body weight, produces a definite red coloration of bone.

TABLE I.

Drug	Electric charge	Diffusion in agar mm in 16 hr	Solubility Ca salt	Adsorption by Ca phosphate	Fixation in mineral matter of bone	Time required to obtain fixation
Congo Red	—	10	insoluble	++	none	—
Alizarine Red	—	17	”	++	fixed	<30 min
Purpurine	—	16	”	++	”	<30 ”
Trypan Blue	—	15	soluble	+	none*	—
Na Bi anthra quinone sulfonate	—	16	insoluble	++	fixed	<30 min
Oxalates	—	>20	”	++	”	not established
Fluorides	—	>20	”	++	”	<35 min[2]
Methylene Blue	+	10		—	none†	—

++ = irreversible; + = reversible; — = non-adsorbed.

* Fixed in the organic matrix of the bone in growing animals. Blotevogel, W., *Zell. and Gewebelehre*, 1924, I/**5**, 601.

† Fixed in the bone cells in frog. Mitrophanow, P. J., *Biol. Zentralblatt*, 1889, **9**, 541.

[2] Volker, J. F., and Sognnaes, R. F., and Bibby, B. G., *Am. J. Phys.*, 1941, **132**, 707.

This coloration was evident in mice sacrificed 24 and 48 hours after injection, while no coloration of other organs was noticeable. In animals sacrificed 30 minutes after injection, the bone was colored, but dye was found also in blood, liver and spleen.

In Table I, the physico-chemical and osteotropic properties of the above bismuth preparation are compared with that of Alizarine, Purpurine, Congo Red, Trypan Blue, Oxalates, and Fluorides.

From a comparison of the physico-chemical properties of fluoride, oxalate, sulfo-alizarine ions, etc., and the above bismuth compound, it can be assumed that the fixation of the bismuth complex in bone takes place by the same mechanism as reported for these ions, *i. e.*, by the formation of an insoluble complex with calcium phosphate, and simultaneous release of PO_4 ions.[3]

Use of the above bismuth preparation in experimental syphilis in rabbits indicates that it exerts its therapeutic influence after the intravenous injection of 4.5 mg Bi per kg given in 3 divided doses. In mice the tolerated dose by i.v. injection is 2.4 mg Bi per kg compared to 5 mg Bi when given as sodium bismuth tartrate.

13425 P

Electroencephalographic Studies: Slow Activity During Hyperventilation in Relation to Age.

WLADIMIR T. LIBERSON AND HANS STRAUSS. (Introduced by Harry Sobotka.)

From the Neurological Service, Mount Sinai Hospital, New York City.

Previous studies have shown that hyperventilation very easily produces slow activity with frequencies of less than 7 cycles per second (delta activity) in the electroencephalograms of children.[1, 2] However, no systematic research has been undertaken as yet, for studying precisely the relationship between this slow activity and age. Two hundred records taken in the Electroencephalography Laboratory of Mount Sinai Hospital were analyzed for this purpose.

Method. The following groups of patients were examined: (1) idiopathic epilepsy (non-deteriorated), (2) symptomatic epilepsy, excluding patients with brain tumor, (3) non-epileptics with headache, fainting, dizziness, etc., without indication of organic disease of the brain, (4) organic disease of the brain, except where the great amount of spontaneous delta activity made it impossible to estimate changes in delta activity during hyperventilation, (5) patients suffering from a variety of systemic diseases associated with hypoglycemia (Addison's Disease, hepatitis, etc.).

---

[3] Ercoli, N., *Boll. Società Italiana Biologia Sperimentale*, 1939, **14**, 16.

[1] Gibbs, F. A., and Gibbs, E. L., *Atlas of Electroencephalography*, 1941, p. 88.

[2] Brill, N. Q., and Seidemann, H., *Arch. Neurol.*, 1941, **46**, 374.

Hyperventilation was induced for at least 2 minutes immediately after a 20-minute record was taken under standard resting conditions. Some patients were fasting; others were examined 2½ to 3 hours after breakfast. Some patients had their blood sugar examined immediately after the hyperventilation period; others received a sugar injection intravenously (50 ml 50% glucose solution). These patients were hyperventilated before and after the sugar injection. The percentage of time of the record occupied by delta activity (% delta time) was determined in all cases. Three values were determined in such cases in which delta activity was present in the spontaneous records: (1) % delta time during hyperventilation (2 minutes), (2) % delta time before hyperventilation (2 minutes), (3) the difference between (1) and (2). The latent time was determined for each record, representing the time from the start of hyperventilation until the appearance of the first burst of slow waves of high amplitude. The percentage of negative findings (absence of slow waves) as a function of age was determined for each group.

Results. Whatever group is considered and whatever delta index is employed, in spite of the great dispersion of individual data, there is a close relationship between the amount of delta activity during the hyperventilation and the age. With an increase in age, the latent time and the percentage of negative findings increase while the total amount of delta activity decreases. The amount of slow activity decreases with age very rapidly up to 15-18 years. After this age, the change becomes much slower up to the age of 25-30 years. After the age of 35-40 the data related to the patients in Groups (1) and (3) are insufficient to warrant any significant correlation with age. However, the data relating to Groups (2) and (5) show that the amount of delta activity is further reduced with increasing age, even after the 35th year.

The average amount of delta activity after hyperventilation is higher in subjects with idiopathic epilepsy than in subjects of Group (3). This difference, in the greatest number of cases, is due to the spontaneous delta activity of the epileptics. The spontaneous delta activity shown by our group of idiopathic epileptics is related to the age in approximately the same manner as the delta activity during hyperventilation.

There is relatively little difference between data concerning fasting and non-fasting patients. This difference concerns the adults predominantly. No significant relationship was found between the blood sugar and the delta activity in a group of 17 subjects of different ages. However, none of these subjects showed a blood sugar below 60 mg % and only 2 presented values above 100 mg %. After glucose injection the delta activity is suppressed or reduced more in adolescents and adults than in children. Subjects with disturbances of carbohydrate metabolism (hypoglycemia, and particularly Addison's Disease) show a great amount of delta activity during hyperventilation,[3] and a slow decrease of this activity with age. Subjects with symptomatic epilepsy generally show a higher amount of delta activity (with shorter latent time) than patients with idiopathic epilepsy. In symptomatic epilepsy the decrease of the slow activity with advancing age is slower than in Groups (1) and (3). Some subjects more than 40 years of age and suffering from organic cerebral diseases (Group 4), show, during hyperventilation, an unusually high increase in delta activity for their age.

On a semi-logarithmic scale the amount of delta activity was plotted against age for the following groups: 1. spontaneous delta activity in epileptics, 2. induced delta activity in (a) non-epileptics, (b) idiopathic epilepsy, (c) symptomatic epilepsy, (d) patients with hypoglycemia, (e) after glucose injection. All curves obtained converge to the same point corresponding to the maximum possible delta activity (100% delta time) and to the age of about 3 years.

Summary. There is a definite relationship between the age and the amount of slow activity during hyperventilation in epileptic and non-epileptic patients. An analogous relationship to age was found for the spontaneous delta activity of patients with idiopathic epilepsy.

13426

K-Strophanthin-β, K-Strophanthoside, Periplocymarin, and Periplocin.

K. K. Chen, Robert C. Anderson and E. Brown Robbins.
From the Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Ind.

From the pioneer work of Arnaud[1] to the elaborate investigation of Brauns and Clossen,[2] the only crystalline principle believed to occur in the seeds of *Strophanthus kombe* was K-strophanthin. It was Jacobs and Hoffmann[3] who demonstrated the latter to be a mixture of cymarin (K-strophanthin-α) and K-strophanthin-β. Recently, Stoll, Renz, and Kreis[4] corroborated Jacobs and Hoffmann's results and, in addition, reported the isolation of a new glycoside, K-strophanthoside.

---

[3] Engel, G. L., and Margolin, S. G., to be published.

Periplocin is a digitalis-like principle first obtained by Lehmann[5] from the bark of *Periploca graeca*. Jacobs and Hoffmann,[6] by treating the extract of the same plant with the enzyme strophanthobiase, were able to crystallize periplocymarin. Lately, Stoll and Renz[7] isolated periplocin and revised its empirical formula. They also showed that by splitting a molecule of glucose under the influence of strophanthobiase, periplocin yielded periplocymarin.

According to our previous publication,[8] it seems that a glycoside containing one sugar molecule (monoside) such as convallatoxin is more potent than a bioside such as scillaren A, and that a bioside in turn is more potent than a trioside such as digitoxin. If this were the case, on account of the following chemical relationships, K-strophanthin-β would be expected to be more active than K-strophanthoside, and periplocymarin more active than periplocin:

K-strophanthoside ——→ K-strophanthin-β + glucose
↓
Strophanthidin + glucose + cymarose

Periplocin ——→ Periplocymarin + glucose
↓
Periplogenin + cymarose

To test the validity of such a hypothesis, the present investigation was undertaken.

The samples of K-strophanthoside, K-strophanthin-β, and periplocin were generously supplied by Dr. Arthur Stoll, Basel, Switzerland. The specimen of periplocymarin was the same as that used in a previous report.[9] The latter, apparently, had lost its alcohol of crystallization upon standing since there was no decrease in weight

---

[1] Arnaud, A., *Compt. rend. Acad. d. sc.*, 1888, **107**, 179.

[2] Brauns, D. H., and Clossen, O. E., *J. Am. Pharm. A.*, 1913, **2**, 604, 715, 843.

[3] Jacobs, W. A., and Hoffmann, A., *J. Biol. Chem.*, 1926, **67**, 609.

[4] Stoll, A., Renz, J., and Kreis, W., *Helv. Chim. Acta*, 1937, **22**, 1484.

[5] Lehmann, E., *Arch. Pharm.*, 1897, **235**, 157.

[6] Jacobs, W. A., and Hoffmann, A., *J. Biol. Chem.*, 1928, **79**, 519.

[7] Stoll, A., and Renz, J., *Helv. Chim. Acta*, 1939, **22**, 1193.

[8] Chen, K. K., Chen, A. L., and Anderson, R. C., *J. Am. Pharm. A.*, 1936, **25**, 579.

[9] Chen, K. K., Anderson, R. C., and Robbins, E. B., *J. Am. Pharm. A.*, 1938, **27**, 113.

when heated in high vacuum. Stock solutions of 1:1000 with 47.5% of ethyl alcohol by volume were prepared with each substance, and dilutions were made from them in saline.

Two methods of assay were adopted—the U.S.P. frog method[10] and the cat method as described previously.[11] The advantage of employing two methods is the mutual confirmation of results—at least qualitatively. In order to minimize the effect of secular variation, each pair of substances, namely, K-strophanthin-β and K-strophanthoside, and periplocymarin and periplocin, was studied simultaneously in the same lots of cats and frogs, and by the same observers. Furthermore, comparable concentrations of the drugs were used. Thus, a solution of 1:10,000 of K-strophanthin-β and

TABLE I.
Comparison of Median Systolic Doses in Frogs.

Compound	Dose, γ per g	No. in systole / No. of frogs used	SD_{50} ± Standard error, γ per g
	0.60	1/5	
	0.70	1/5	
	0.80	1/10	
	0.90	1/5	
K-Strophanthin-β 1:10,000	1.00	6/15	0.982 ± 0.043
	1.10	12/15	
	1.20	12/15	
	1.30	9/10	
	1.40	9/10	
	1.50	4/5	
	0.80	1/5	
	1.00	2/5	
	1.10	2/10	
K-Strophanthoside 1:10,000	1.20	0/10	1.341 ± 0.099
	1.30	6/10	
	1.40	8/10	
	1.50	3/5	
	2.00	4/5	
	2.00	1/5	
	2.50	0/15	
Periplocymarin 1:4,000	3.00	4/15	3.161 ± 0.115
	3.50	8/10	
	4.00	10/10	
	1.50	0/5	
	2.00	0/15	
	2.25	2/10	
Periplocin 1:4,000	2.50	5/15	2.815 ± 0.112
	3.00	6/10	
	3.50	8/10	
	4.00	5/5	

---

[10] U. S. P. XI, 1936, 397.

[11] Chen, K. K., Anderson, R. C., and Robbins, E. B., *J. Pharm. and Exp. Therap.*, 1940, **69**, 279.

TABLE II.
Comparison of Mean Lethal Doses in Cats.

Compound	Cat No.	Sex	Body wt, kg	Heart wt, g	Fatal dose by Body wt, γ per kg	Fatal dose by Heart wt, γ per g	Mean lethal dose ± standard error Body wt, γ per kg	Mean lethal dose ± standard error Heart wt, γ per g
K-Strophanthin-β	2	F	2.200	8.0	132.7	36.5	128.2 ± 3.1	33.4 ± 0.5
	3	F	1.916	7.8	132.3	32.5		
	7	M	2.049	7.9	124.9	32.4		
	8	F	2.302	9.1	127.3	32.2		
	11	F	2.652	9.9	127.1	34.0		
	12	M	2.150	8.7	134.0	33.1		
	13	M	2.007	8.0	139.5	35.0		
	16	M	2.657	9.5	112.9	31.6		
	19	M	2.362	8.1	113.5	33.1		
	20	M	2.198	9.2	141.9	33.9		
K-Strophanthoside	1	F	1.700	6.6	210.6	54.2	186.5 ± 11.1	50.8 ± 2.9
	4	F	2.363	8.6	191.7	52.7		
	5	M	2.681	8.1	158.1	52.3		
	6	F	2.263	7.5	218.7	66.0		
	9	F	2.630	10.7	141.1	34.7		
	10	M	1.884	7.9	246.0	58.7		
	14	F	2.243	9.2	195.7	47.7		
	15	M	2.560	8.5	142.2	42.8		
	17	F	2.270	8.1	176.7	49.5		
	18	F	2.159	8.2	213.1	56.1		
Periplocymarin	27	M	1.972	6.7	133.9	39.4	156.1 ± 10.5	40.3 ± 2.7
	29	M	2.235	9.6	112.8	26.2		
	32	F	2.509	10.4	157.8	38.1		
	33	F	2.756	8.6	112.1	35.9		
	36	M	2.136	6.8	163.4	51.3		
	37	F	2.349	9.3	212.8	53.8		
	39	F	2.490	9.2	185.5	50.2		
	40	M	2.287	9.6	177.5	42.3		
	41	F	2.133	8.7	155.2	38.0		
	42	F	2.524	12.6	180.3	36.1		
Periplocin	23	F	2.746	10.2	123.1	33.1	120.5 ± 6.0	31.7 ± 1.3
	24	F	2.403	10.1	126.3	30.0		
	25	F	2.030	8.7	127.6	29.8		
	26	F	2.212	8.5	140.8	36.6		
	28	F	2.674	9.3	114.8	33.0		
	30	F	2.093	7.9	116.6	30.9		
	31	F	2.257	9.3	114.5	27.8		
	34	M	2.752	8.2	91.2	30.6		
	35	M	2.695	10.6	102.8	26.1		
	38	F	2.469	9.6	161.2	41.5		

K-strophanthoside, and one of 1:4000 of periplocymarin and periplocin, were administered to frogs; and a dilution of 1:100,000 of each of the 4 substances was injected intravenously into cats at the rate of 1 cc per minute.

The results are summarized in Tables I and II. As postulated, K-strophanthin-β, a bioside, proves to be more potent than K-strophanthoside, a trioside, by the comparison of the median systolic doses in frogs, and the mean (geometric) lethal doses in cats based on either body weight or heart weight. The differences are highly significant statistically. On the other hand, periplocin, a bioside, shows a tendency of having a stronger action than periplocymarin, a monoside. The difference in frogs approaches significance at the 5% point, and in cats the difference is definitely significant. The median emetic doses in pigeons by intravenous injection of periplocin and periplocymarin showed a similar relationship as disclosed in a previous note.[11]

The above data clearly indicate that the cardiac action of a glycoside does not necessarily decrease as the number of sugar molecules increases. The position of linkage, perhaps, partly determines the potency of the product. It will require an investigation of many other compounds of the same type before any generalization can be safely made.

Summary. K-Strophanthin-β is more potent than K-strophanthoside in frogs and cats, while periplocymarin is less potent than periplocin. The decrease in the number of sugar molecules of a cardiac glycoside, therefore, may or may not be accompanied by an increase in activity.

13427

Substituted Sulfanilamidopyrimidines.*

EDWIN J. FELLOWS.

From the Department of Pharmacology, Temple University School of Medicine, Philadelphia, Pa.

Experimental[1, 2] and clinical[3, 4, 5] bacterial chemotherapeutic activity has been reported for 2-sulfanilamidopyrimidine (sulfadiazine). Modification of the pyrimidine portion of this compound, without concurrent loss of chemotherapeutic effectiveness, appears possible

---

* This investigation was supported in part by the D. J. McCarthy Foundation.

[1] Roblin, R. O., Williams, J. H., Winnek, P. S., and English, J. P., *J. Am. Chem. Soc.*, 1940, **62**, 2002.

[2] Feinstone, W. H., Williams, R. D., Wolff, R. T., Huntington, E., and Crossley, M. L., *Bull. Johns Hopkins Hosp.*, 1940, **67**, 427.

[3] Flippin, H. F., Rose, S. B., Schwartz, L., and Domm, A. H., *Am. J. Med. Sci.*, 1941, **201**, 585.

[4] Finland, M., Strauss, E., and Peterson, O. L., *J. A. M. A.*, 1941, **116**, 2641.

[5] Dingle, J. H., Thomas, L., Morton, A. R., *J. A. M. A.*, 1941, **116**, 2666.

from the report[1] that the protective power of the 4-methyl derivative was comparable with that of its parent, sulfadiazine. A number of substituted sulfanilamidopyrimidines (Table I) prepared by Caldwell, Kornfeld and Donnell[6] were tested for protective action in the present studies[†] in mice infected with a 10^{-6} dilution of an 18-hour serum broth culture[‡] of β-hemolytic streptococcus. This infective dose of 100-1000 MLD's always was injected intraperitoneally in 0.5 cc of serum broth. Therapy consisted of administration of a 10 mg dose of a given compound in 0.5 cc of 10% acacia solution by stomach tube 30 minutes after infection and once daily for 2 days thereafter. All of 90 control mice died within 48 hours after infection (Table I). Compounds III, IV and V were inactive and I exhibited only a slight protective action, whereas a definite prolongation of survival time was observed after sulfadiazine,[§] VI and II (Table I). While sulfadiazine and VI gave almost complete protection, 50% of the mice receiving II died during the period of therapy (Table I). The 10-day survival rate after sulfadiazine, compounds VI and II was 22.5, 10.0 and 2.5% respectively (Table I).

Derivative VI by mouth killed 10/10 mice in doses of 5.0 g/kg, 9/10 in 2.5 g/kg and 5/10 in 1.5 g/kg doses. No deaths were observed after sulfadiazine in doses up to 5.0 g/kg in the present studies and survival of all mice receiving doses up to 10.0 g/kg of this compound previously has been reported.[2] It would appear, therefore, that VI, the most effective antistreptococcic agent in the present group of substituted sulfanilamidopyrimidines is unworthy of extended study because of its relatively high oral toxicity in mice.

Summary. 2-sulfanilamido-5,6,7,8-tetrahydroquinazoline (I), 2-sulfanilamido-4,5-dimethyl pyrimidine (II), 2-sulfanilamido-bornylene pyrimidine (III), 2-sulfanilamido-4-n-hexylpyrimidine (IV), 2-sulfanilamido-5,6,7,8-tetrahydro-8-isopropyl-5-methyl quinazoline (V) and 2-sulfanilamido-4,6-dimethyl pyrimidine (VI) were tested for chemotherapeutic activity in mice infected with strain C-203 of β-hemolytic streptococcus. Compounds III, IV and V were inactive and I exhibited only a slight protective action. A

---

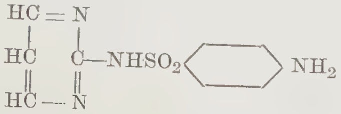
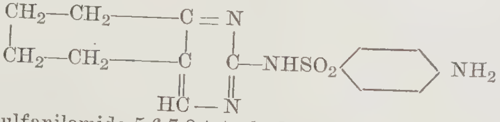
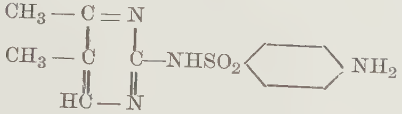
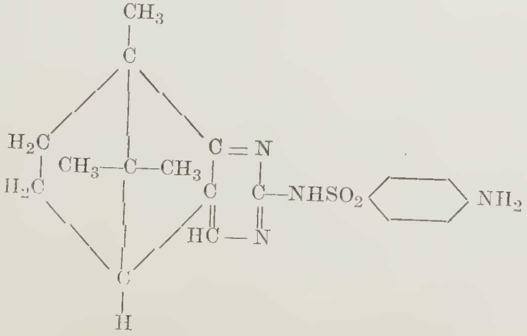
[6] Caldwell, W. T., Kornfeld, E., and Donnell, C. K., *J. Am. Chem. Soc.*, 1941, **63**, 2188.

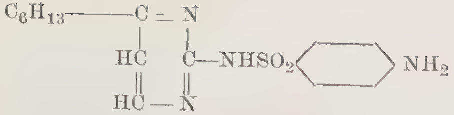
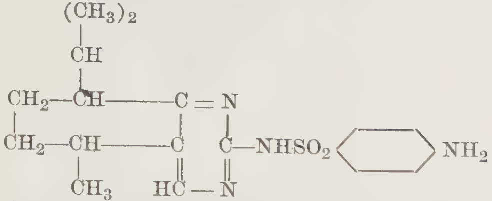
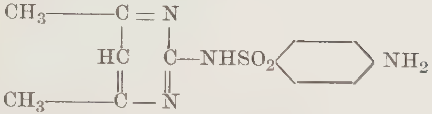
[†] Samples of the substituted sulfanilamidopyrimidines used in the present experiments were made available by Dr. William T. Caldwell of the Department of Organic Chemistry, Temple University.

[‡] Culture C-203 was made available through the courtesy of Dr. E. K. Marshall, Jr.

[§] The sulfadiazine used in the present studies was supplied through the courtesy of Dr. David A. Bryce of the Calco Chemical Company.

TABLE I.
Chemotherapeutic Effectiveness in Hemolytic Streptococcal Infection of Mice.

	Compound	No. of mice	No. of deaths (Days after infection)										Survival %
			1	2	3	4	5	6	7	8	9	10	
	None (Controls)	90	87	3									0
	 2-sulfanilamidopyrimidine (Sulfadiazine)	40	0	0	0	5	13	4	4	4	1	0	22.5
(I)	 2-sulfanilamido-5,6,7,8-tetrahydroquinazoline	35	16	15	1	1	1	1					0
(II)	 2-sulfanilamido-4,5-dimethyl pyrimidine	40	3	13	4	7	5	4	3	0	0	0	2.5
(III)	 2-sulfanilamidobornylene pyrimidine	35	35										0

(IV)	 2-sulfanilamido-4-n-hexylpyrimidine	35	35										0
(V)	 2-sulfanilamido-5,6,7,8-tetrahydro-8-isopropyl-5-methyl quinazoline	35	35										0
(VI)	 2-sulfanilamido-4,6-dimethyl pyrimidine	40	0	2	0	5	11	6	6	6	0	0	10.0

definite prolongation of survival time was caused by II and VI, however, neither of these appeared worthy of extended study because II failed to afford complete protection during therapy and VI exhibited a relatively high acute oral toxicity in mice.

I wish to express my thanks and appreciation to Dr. A. E. Livingston for encouragement and helpful suggestions.

13428

Graying of Hair Produced by Ingestion of Phenylthiocarbamide.

C. P. Richter and K. H. Clisby.

From the Psychobiological Laboratory, Phipps Psychiatric Clinic, Johns Hopkins Hospital.

The results of previous experiments demonstrated the high degree of toxicity of the bitter tasting phenylthiocarbamide for rats (Richter and Clisby[1]), 1 to 2 mg usually being lethal. With these and slightly larger doses the rats died within 3 to 18 hours with one outstanding symptom, pleural edema and effusion. In some rats from 10 to 14 cc of a clear serous fluid accumulated in the thoracic cavity within only a few hours' time. By administering the drug in sublethal doses for a few days and then gradually increasing the doses over a period of several months, we succeeded in increasing the tolerance to 18 mg per day in some animals. At autopsy rats treated for several months did not show any thoracic changes, but all of them demonstrated marked hyperplasia and hyperactivity of the thyroid gland. Histologically such a gland had all the characteristics of an exophthalmic goiter. Two of these rats which originally had been solid black became gray over a saddle blanket area on the back. The observations on this effect of the drug were limited to these 2 rats since the other rats used for the experiments were either white or tan hooded.

Further observations have now been undertaken to determine (1) the consistency with which feeding phenylthiocarbamide causes the hair to turn gray, (2) the constancy of the gray hair patterns which it produces, and (3) whether after the phenylthiocarbamide is no longer given the hair returns to normal.

Including the 2 rats from the previous experiments we have a group of 23 black rats which received phenylthiocarbamide for periods which ranged from 41 to 125 days. Each rat was kept in a separate cage containing a food cup with the stock diet and a graduated inverted 100 cc bottle with the phenylthiocarbamide solution. The concentration of the solution was started at 0.001% and increased at intervals of approximately 12 days as follows: 0.01%, 0.02%, 0.04%, 0.06%, 0.08%, 0.1%. During the experiment the actual amount of phenylthiocarbamide received by the rats per day changed from 1.3 to 14.1 mg. Thus, in order to satisfy their thirst, the rats were forced to take a certain amount of the drug at frequent intervals throughout the day and night.

---

[1] Richter, C. P., and Clisby, K. H., *Arch. Pathol.*, in press.

With one exception the 23 rats showed definite graying after they were started on the phenylthiocarbamide. In the one exception the hair showed patterns of slightly different degrees of blackness but no definite graying. Graying of hair was first noticed as soon as 27 days in some of the rats, though it may have been present earlier and escaped notice. In most instances it was very distinct after 58 days on the phenylthiocarbamide solution.

All except 3 of the rats showed essentially the same pattern of graying, which developed progressively during the period of treatment. The hair first turned gray in back of a line which paralleled that of the ears at a distance of about 1 cm. (See Fig. 1-A.) In front of this line the hair remained black. Progressively with the increased dosage and length of time of treatment, the graying process moved along the back until it reached the lumbar region where a clear-cut pattern in the shape of a saddle blanket manifested itself. (See Fig. 1-B.) Later the graying involved all of the hair down to the tail. (See Fig. 1C.) At the same time that the graying process descended along the back, it proceeded forward under the ear and eyes and included most of the snout. In all instances, however, the top of the head remained black. (See Fig. 2-A.) It is probably significant that in human beings also the hair on the top of the head

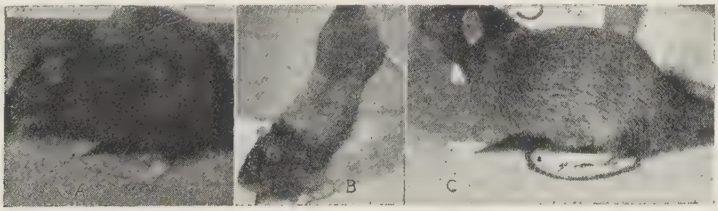


Fig. 1.

Photograph showing pattern and location of gray hair at 3 typical stages of graying in the phenylthiocarbamide-treated rats.

A. Gray hair starting on transverse line about 1 cm back of ears.

B. Saddle blanket shaped area on back which appeared later.

C. Final stage of graying. Note that hair on top of head still is black.

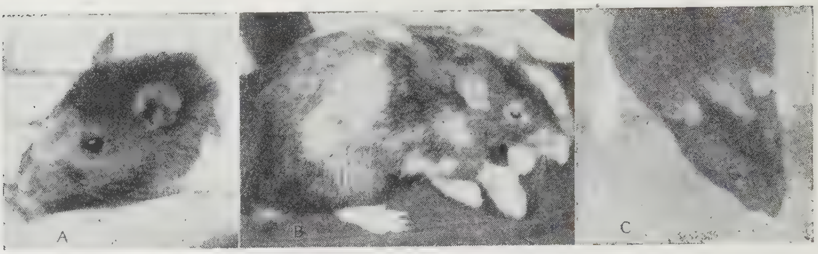


FIG. 2.

A. Facial pattern of graying in a black hooded rat. The gray pattern included the area between the eyes and below a longitudinal line which ran just below the eyes and ears.

B. Graying with irregular spots of white hair on different parts of the body found in three rats.

C. Pattern of brownish red area of hair found between the ears in a rat which had been well grayed but which for over a month had not received any phenylthiocarbamide.

is the last to turn gray. As is well known, the graying process usually starts at the temples and gradually works up to the top of the head.

The 3 rats which formed exceptions to the more usual pattern had irregularly shaped areas of white hair on different parts of the body. (See Fig. 2-B.)

In answer to the third question—does the hair return to normal after cessation of the drug?—five of the rats were given tap water in place of the phenylthiocarbamide solution after 41-91 days on the drug. In each of these animals the hair turned black again over the entire surface of the body, the return of color requiring approximately 83 days and taking place in reverse order to the graying process; that is, the hair along the line back of the ears was the last to return to normal. There was usually an intermediate stage during which the hair had a distinctly brownish cast, and it was noticed that in 3 rats there were sharply defined but irregular patches of a brownish red color between the ears—these too turned black again within the 90-day period. Fig. 2-C shows a photograph of such an area.

The graying areas found in most of the rats did not follow, so far as we were able to determine, any known anatomical or developmental pattern of hair coloring, except that the line over the shoulders at which the graying started coincided fairly closely with the lower edge of the hood in hooded rats. These patterns differed quite markedly from those produced by Morgan and Simms[2] in rats with diets which lacked one or two of the filtrate factors. None of our rats showed the dermatitis. The general appearance of the rats grayed by the two methods is quite different.

---

[2] Morgan, A. F., and Simms, H. D., *J. Nutrition*, 1940, **19**, 233.

The results of our experiments indicate that graying of the hair may be produced by a positive factor in the diet as well as by the lack of some factor, such as pantothenic acid. It is possible that the phenylthiocarbamide may combine with the pantothenic acid or some other factor in the body and thus produce an actual deficiency of the latter.

These experiments bring more evidence for the close relationship between graying of the hair and the activity of the thyroid gland. However, the fact that graying was found in several animals in which supposedly all of the thyroid gland had been removed indicates that the phenylthiocarbamide probably has a more direct effect on the hair.

Summary. (1) After being placed on a stock diet and given phenylthiocarbamide solution as the sole source of fluid, with one exception 23 black or black hooded rats showed definite graying, some as early as 27 days and all within 58 days. (2) Approximately 83 days after the phenylthiocarbamide solution was replaced by tap water, five rats that had shown marked graying turned completely black again. (3) The experiments showed that graying may result from a positive factor in the diet, a poison, as well as from a deficiency of some factor.

13429

A Phosphorylase in Calcifying Cartilage.*

ALEXANDER B. GUTMAN AND ETHEL BENEDICT GUTMAN.

From the Department of Medicine, Columbia University, and the Presbyterian Hospital, New York City.

It has been shown by histochemical[1, 2, 3] and chemical[3, 4] methods that cartilage cells preparatory to calcification accumulate large stores

---

* The work reported in this communication was carried out under the John A. Hartford Foundation Gift.

[1] Creighton, C., *Microscopic Researches on the Formative Property of Glycogen,* Adam and Charles Black, London, 1896.

[2] Harris, H. A., *Nature,* 1932, **130**, 996.

[3] Glock, G. E., *J. Physiol.*, 1940, **98**, 1.

[4] Hoffman, A., Lehmann, G., and Wertheimer, E., *Arch. f. d. ges. Physiol.*, 1928, **220**, 183.

of glycogen, which disappear abruptly just prior to or during the early course of calcium deposition. The processes of glycogen depletion and of calcification appear to be closely coördinated, suggesting some inter-relation;[1, 2] for example, phosphoric esters formed during glycogen breakdown might serve as substrates for bone phosphatase.[2, 3, 4, 5] This inherently plausible hypothesis, as yet unsupported by direct evidence, has been largely ignored in current speculation concerning the mechanisms of calcification.

In liver and muscle, according to recent studies by Cori,[6] Parnas[7] and others, the major pathway of glycogen degradation involves phosphorolysis and the formation of intermediate phosphoric esters. The glycogenolytic cycle is initiated by the reaction

$$\text{Glycogen} + \text{inorganic phosphate} \rightleftharpoons \text{glucose-l-phosphate}$$

which is catalyzed by an enzyme, phosphorylase, shown to be present in liver, muscle, and certain other tissues. Evidence is offered here that a similar phosphorylating enzyme system, associating glycogenolysis with calcification, occurs in calcifying cartilage.

Methods. The proximal and distal ends of the femora, tibiae and humeri of young rabbits (500-800 g) or rats (20-25 days) were used, after careful removal of all muscle and most shaft bone. The tissue was rapidly weighed, minced and ground, then transferred to glass-stoppered centrifuge tubes. These, in experiments with tissue pulp, contained a 1:1 proportion of digest mixture prepared as indicated in Table I (1.0 cc was used where the tissue weighed less than 1.0 g), with which the pulp was quickly stirred. After brief centrifugation, duplicate control samples were removed from the supernatant with a Lang-Levy micropipette. The digest was then mixed and allowed to stand, samples being taken at intervals after preliminary centrifugation.

In experiments in which not tissue pulp but tissue extracts were used, the centrifuge tubes contained only distilled water equal in volume to the tissue used, but not less than 1.0 cc. After stirring 15 minutes, then centrifuging, the supernatant extract was added to digest mixtures (prepared so as to give proper final concentrations), as described.

Serial samples obtained at intervals during digestion were discharged into 1.0 cc 5% trichloracetic acid. After standing 10 minutes, 9.0 cc water was added, and the inorganic P content of filtrate

---

[5] Robison, R., and Rosenheim, A. H., *Biochem. J.*, 1934, **28**, 684.

[6] Cori, C. F., and Cori, G. T., *Ann. Rev. Biochem.*, 1941, **10**, 151.

[7] Parnas, J. K., *Glykogenolyse, Handbuch d. Enzymologie* (edited by Nord and Weidenhagen), Akad. Verlagsgesellschaft, Leipzig, 1940, **2**, 902.

aliquots determined by the Kuttner-Lichtenstein method,[8] using an Evelyn photoelectric colorimeter. The percentages of inorganic P and reducing sugar recoverable after hydrolysis of digest filtrates were determined in some samples after boiling with N sulfuric acid for 7 and 30 minutes.

As indicated in the accompanying tables, application of this semi-microtechnic to liver pulp and extracts yields results in satisfactory agreement with those recorded for liver by Ostern, Herbert and Holmes[9] and by Cori, Cori and Schmidt.[10]

Results. When suitable concentrations of glycogen and inorganic P were added to ground epiphyses of young animals, the inorganic P content of the digest decreased rapidly (Table I). We did not identify the phosphoric esters formed beyond establishing that no

TABLE I.

Effect of Young Rabbit Liver and Epiphyseal Pulp on Inorganic P Content of Phosphate-glycogen Mixtures; Influence of Temperature and Fluoride on the Reaction.

				Liver pulp Inorg. P		Epiphyses pulp Inorg. P	
Exp.	Additions* to tissue pulp	Temp., °C	Time, (min.)	mg/g tissue	% change	mg/g tissue	% change
1	P,G,F	37	2	1.62	0	1.13	0
			15	0.54	—67	0.85	—25
			30	0.47	—71	0.75	—34
			60	0.38	—76	0.61	—46
			120	0.52	—68	0.57	—50
2a	P,G,F,	22	2	1.38	0	1.04	0
			15	0.74	—50	0.84	—19
			30	0.45	—67	0.74	—29
			60	0.22	—84	0.64	—38
			120	0.20	—85	0.62	—40
2b	P,G	22	2	1.52	0	1.03	0
			15	1.18	—22	0.83	—19
			30	1.16	—24	0.73	—29
			60	1.14	—25	0.68	—34
			120	1.37	—10	0.59	—43
3	P,G,F	15	2	1.46	0	1.09	0
			15	1.03	—29	0.96	—12
			30	0.74	—49	0.91	—17
			60	0.41	—72	0.87	—19
			120	0.28	—81	0.84	—23

*P—M/45 phosphate buffer at pH 7.2.
G—0.4% glycogen.
F—M/40 NaF.
(All concentrations indicated signify those in final digest mixture.)

---

[8] Bodansky, A., *J. Biol. Chem.*, 1932, **99**, 197.

[9] Ostern, P., Herbert, D., and Holmes, E., *Biochem. J.*, 1939, **33**, 1858.

[10] Cori, G. T., Cori, C. F., and Schmidt, G., *J. Biol. Chem.*, 1939, **129**, 629.

significant inorganic P could be recovered after 7 minutes' hydrolysis, even in the presence of fluoride, and that a variable amount, sometimes over 50%, reappeared after 30 minutes' hydrolysis.

Esterification was less marked than observed with liver pulp but the activity of the two tissues in relation to time and temperature was similar (Table I). As with liver, the disappearance of inorganic P was inhibited by M/100 phlorizin. A striking difference, however, was observed with M/40 NaF which, in contrast with its marked effect on liver, caused no change in the rate of disappearance of inorganic P by ground epiphyses (Table I).

Whereas with tissue pulp, addition of muscle adenylic acid was found to be unnecessary, aqueous extracts of the epiphyses of young animals showed no phosphorylase activity in the presence of glycogen and inorganic P unless or until the coenzyme was added (Table II). This behavior corresponds with that recorded for liver and muscle phosphorylase.[9, 10]

When glucose-l-phosphate was added to aqueous extracts of young epiphyses in the presence of catalytic amounts of glycogen and muscle adenylic acid, an increase in the inorganic P content of the digest was observed (Table II). An iodine test for glycogen after several hours gave a faint brownish color which gradually faded.

Discussion. The representative results cited were not obtained with isolated hypertrophic cartilage but with whole epiphyses, which

TABLE II.

Effect of Aqueous Extracts of Young Rabbit Epiphyses on Inorganic P Content of Phosphate-glycogen Mixtures, with and without Added Muscle Adenylic Acid; and on Glucose-l-phosphate. (Temp. 22°C.)

Exp.	Additions to tissue extracts	Time (min.)	Inorganic P	
			mg/g tissue	% change
4a	M/25 phosphate buffer pH 7.2, 0.5% glycogen, M/25 NaF and muscle adenylic acid	2	.83	0
		15	.67	—19
		30	.64	—23
		60	.61	—27
		120	.64	—23
4b	Same but adenylic acid omitted	2	1.19	0
		15	1.18	0
		30	1.18	0
		60	1.19	0
		120	1.17	0
4c	Adenylic acid now added to 4b	15	.69	—39
5	M/200 glucose-l-phosphate, traces of glycogen and adenylic acid	1	.18	
		30	.41	
		60	.58	
		240	1.52	

include bone, marrow, blood, small-cell cartilage, etc. Part of the phosphorylase activity noted could be ascribed to the hypertrophic cartilage present, however, since this, when dissected out as cleanly as possible from young rat epiphyses, produced a 17% decrease in the inorganic P content of phosphate-glycogen digests in one hour. The epiphyses of adult rats, the persistent plate of which contains only small-cell cartilage, also show definite phosphorylase activity although articular (non-calcifying) cartilage has no significant effect, according to Lutwak-Mann.[11] This may be due, in part, to the presence of osseous tissue since we found distinct activity in isolated cortical bone from the shaft of both young and mature rats.

The results cited in Tables I and II imply a very rapid rate of reaction of the enzymes involved. This contrasts with the extremely slow reverse catalytic effect of bone phosphatase. In fact, the dephosphorylating action of aqueous extracts of young rabbit epiphyses was found to be slight at pH 7.2 and 22° : 0.8 mg inorganic P was split off from β-glycerophosphate substrate whereas 19.4 mg were hydrolyzed by the same extract at pH 8.6 and 37°. Activation by adenylic acid further indicates that our results cannot be ascribed wholly to bone phosphatase.

We conclude that there appears to be present in calcifying cartilage a phosphorylative glycogenolytic enzyme system capable of synthesizing potential substrates for bone phosphatase at zones of calcification before blood sources become available, and for supplementing those blood sources after they become available. Inorganic phosphate for calcification can be formed from organic esters not only by bone phosphatase but by phosphorylase.

If glycogenolysis is a prerequisite for calcification in cartilage, it becomes clear why calcification *in vitro* is blocked by glucose, fluoride and iodoacetate[5] in amounts too small to inhibit bone phosphatase or to affect the solubility of bone salts. The inhibitory effects of glucose, fluoride and iodoacetate further make it unlikely that dephosphorylation of glucose-l-phosphate by bone phosphatase (which we find takes place readily *in vitro* at pH 8.6) affords the chief source of inorganic P for calcification; moreover, glucose-l-phosphate appears to be converted to other phosphoric esters too rapidly to serve as the chief substrate for bone phosphatase.

---

[11] Lutwak-Mann, C., *Biochem. J.*, 1940, **34**, 517.

13430

Effect of Various Dietary Supplements on Growth and Lactation in the Albino Mouse.*

Zelda B. Ball and Richard H. Barnes.

From the Divisions of General Physiology and Physiological Chemistry, Department of Physiology, University of Minnesota, Minneapolis.

In the studies of the specific requirements of vitamins of the B complex for normal lactation it has been concluded that thiamin,[1] riboflavin,[2] pantothenic acid,[3] factor W,[4] the filtrate fraction,[5] and vitamins L_1 and L_2[6] are necessary for normal lactation in the rat. Some of the recently developed strains of brewer's yeast have been claimed good sources of all the above mentioned vitamins, and it is a well known fact that purified diets containing an adequate amount of yeast will provide for normal reproduction and lactation in the rat.

All of these studies have been conducted on rats and, while it is well known that mice are very difficult animals to raise, there is essentially nothing known about specific dietary factors needed for lactation in this species. The present communication is the first of a group of studies on specific dietary requirements of the mouse.

Four groups of 25 Strain A female mice each were placed at weaning on the purified diets A, B, C, and D shown in Table I. A fifth group was given a commercial food widely used in small animal colonies (Purina Fox Chow), and a sixth was fed this same commercial food together with daily supplements of fresh lettuce leaves and dried brewer's yeast. The 4 purified diets were made in such a manner that the protein, fat, and carbohydrate contents were the same for each diet. Furthermore, in the diets containing liver extract and dehydrated grass† (C and D) the amount of yeast was decreased so that with a margin of safety these diets did not contain

---

* Aided by the Sivertsen Foundation for Cancer Research.

Assistance in the preparation of these materials was furnished by the personnel of Work Projects Administration Official Project 65-1-71-140, Sub-Project 363.

[1] Evans, H. M., and Burr, G. O., *J. Biol. Chem.*, 1928, **76**, 263.

[2] Haussemann, D. L., and Hetler, R. A., *J. Nutrition*, 1931, **4**, 127.

[3] Jukes, T. H., Proc. Soc. Exp. Biol. and Med., 1940, **45**, 625.

[4] Sure, B., and Beach, A., *J. Nutrition*, 1940, **19**, 57.

[5] Morgan, A. F., and Sims, H. D., *Science*, 1939, **89**, 565.

[6] Nakahara, W., Inukai, F., and Ugami, S., *Sci. Papers Inst. Phys. Chem. Research*, 1939, **36**, 327.

† We are indebted to the Cerophyl Laboratories for a generous supply of this material.

TABLE I.
Percentage Composition of Diets.

Ingredient	Experiment 1				Experiment 2			
	A	B	C	D	A	B	C	D
Glucose	34	34	34	30.5	19	19	19	15.5
Casein	31	31	31	28.5	28	28	28	25.5
Lard	20	20	20	19.5	19	19	19	18.5
Yeast*	8	8	6	5.7	8	8	6	4.7
Salt mixture†	7	7	7	5.8	6	6	6	5.8
Ascorbic acid		0.1				0.1		
Liver extract‡			2				2	
Dehydrated grass§				10				10
Wheat bran					20	20	20	20

*Anheuser Busch, Strain G.
†Hubbel, R. B., Mendel, L. B., and Wakeman, A. J., *J. Nutrition*, 1937, **14**, 273.
‡Liver fraction B, Wilson Laboratories, Chicago.
§Cerophyl, Cerophyl Laboratories, Kansas City, Mo.

more thiamin, riboflavin, or pantothenic acid than the basal diet A. The mice in the 6 groups were placed 5 to a cage. Those mice receiving the purified diets were given weekly supplements of 0.02 cc each of cod liver and wheat germ oil. Food consumption was measured daily and the mice were weighed every other day. At approximately 100 days of age the mice were bred and the resulting pregnant females were individually caged. Careful litter records were kept through 2 successful pregnancies. At the end of this period the diets were altered so as to contain 20% wheat bran (Table I, Experiment 2). Mice receiving these 4 diets were bred and litter records again kept through 2 successful pregnancies.

The growth rates of the mice receiving the 4 purified diets (Experiment 1) were definitely greater than mice on either the commercial food alone, or the commercial food supplemented with lettuce and yeast. Ascorbic acid in the pure form or as a component of the dehydrated grass had no effect on growth rate or food intake.

Litter records are presented in Table II. Several observations appear to be significant. The basal purified diet is definitely inadequate for lactation. In the absence of wheat bran addition of ascorbic acid or liver extract does not increase the survival of mice through the suckling period. Both grass and wheat bran materially improve the diet for lactation and their effects may be additive. The addition of both ascorbic acid and wheat bran to the basal diet seems to have a greater effect than wheat bran alone.

Wheat bran is very rich in phytin, and as this compound has recently been shown to be a dietary essential for the mouse,[7] feeding experiments with both inositol and phytin were carried out to see

---

[7] Wooley, D. W., *Science*, 1940, **92**, 384.

TABLE II.
Litter Record of Mice.

Group diet	No. litters	No. litters weaned	% litters weaned	No. mice born	No. mice weaned	% mice weaned	Avg No. mice per litter
		Experiment 1.					
A Basal	37	3	8.1	188	5	2.7	5.1
B ’’ + Ascorbic acid	39	1	2.6	181	1	0.6	4.6
C ’’ + Liver extr.	42	1	2.4	226	2	0.9	5.4
D ’’ + Grass	41	12	29	240	45	18.8	5.9
Commercial diet	41	27	66	246	135	55	6.0
’’ ’’ (supplemented)	39	32	82	247	155	63	6.3
		Exper. 2 (Added Wheat Bran).					
A Basal	31	11	35	142	33	23	4.6
B ’’ + Ascorbic acid	29	11	38	116	42	36	4.0
C ’’ + Liver extr.	41	13	32	217	44	20	5.3
D ’’ + Grass	33	17	52	187	75	40	5.7
		Exper. 3 (Adults Started on Diets).					
Basal	17	7	41	88	22	25	5.2
’’ + Inositol	15	2	13	71	6	8.5	4.7
’’ + Phytin	15	3	20	86	8	9.3	5.7

if either of these substances was the active constituent of the wheat bran. Three groups of 10 adult female mice each were placed on the basal purified diet. One of these groups received 100 mg of inositol per 100 g of diet and a second group received 100 mg of phytin per 100 g of diet.

The weaning record of approximately 2 litters per mouse is shown in Table II, Experiment 3. Neither of the 2 supplements enhanced lactation. On the contrary, it would appear that both inositol and phytin in the amounts administered had a deleterious effect. In contrast to Experiment 1, the mice used in this study had been raised to maturity on the commercial diet and were then placed on the basal purified diet. They were bred immediately following the change of diet. It will be seen in Table II that the lactation record of the controls in Experiment 3 was superior to the controls in Experiment 1 that had received the same diet from the time of weaning. This is perhaps due to the storage of certain dietary essentials for lactation that were not entirely depleted during the period of gestation when the mice in Experiment 3 were receiving the inadequate basal diet.

It is well recognized that ascorbic acid is not necessary in the diet of the rat. This has been assumed to be true for the mouse, but Kleiner and Tauber[8] have reported a few observations suggesting that the addition of small supplements of ascorbic acid to the diet of mice results in an increased growth rate. Such an effect of ascorbic acid is not substantiated by the results of the present study.

---

[8] Kleiner, I. S., and Tauber, H., *Food Research*, 1936, **1**, 399.

Essentially the only observation in the literature regarding specific vitamins necessary for lactation in the mouse is that of Nakahara, *et al.*[9] These investigators came to the conclusion that vitamins L_1 and L_2, which are supposed to be necessary for lactation in rats, are also necessary for mice but that mice are more resistant to the development of L_1 and L_2 deficiency than rats. Furthermore, they state that brewer's yeast is an adequate source of these two lactation vitamins. Contrary to the above mentioned investigation, the results of the present study show that diets containing 8% brewer's yeast are completely inadequate for lactation in the mouse. However, 2 supplements, namely dehydrated grass, and wheat bran will effectively increase lactation of mice raised on a purified diet containing brewer's yeast.

According to calculations of the amounts of thiamin, riboflavin, and pantothenic acid in the yeast (Anheuser Busch Strain G[‡]), dehydrated grass (cerophyl[§]), and wheat bran[10, 11] used in these studies, the level of these substances in the diets with dehydrated grass and wheat bran supplements was not higher than in the basal diet. It is concluded that some other component of grass and bran exerts the favorable effect upon lactation. This substance is not inositol or phytin, because when added these compounds did not improve lactation. Furthermore, as the addition of a liver preparation that has been used by other investigators as a potent source of factor W did not result in an increased lactation, it would seem probable that this factor is not of concern in interpreting the results of the studies presented here. It would appear likely that mice either require more of some lactation promoting substance than do rats, or that they require some substance not necessary in the diet in rats.

Summary. On the basal diets studied, ascorbic acid has been shown to have no influence on the growth rate of mice. Purified diets containing 8% yeast are not adequate for lactation in mice. Both dehydrated grass and wheat bran effectively increase lactation, but these two substances when added to a basal purified diet alone or combined do not provide for as good lactation as does a stock commercial diet. It has been concluded that the lactation-promoting effect of the dehydrated grass and wheat bran is not due to an increase of thiamin, riboflavin, pantothenic acid, factor W, or inositol in the diet.

---

[9] Nakahara, W., Inukai, F., Ugami, S., and Takahashi, K., *Sci. Papers Inst. Phys. Chem. Research*, 1939, **36**, 318.

[‡] Personal communication from Anheuser Busch, Inc.

[§] Personal communication from Cerophyl Laboratories.

[10] Fixsen, M. A. B., and Roscoe, M. H., *Nutrition Abs. and Rev.*, 1937, **7**, 823.

[11] Jukes, T. H., *J. Nutrition*, 1941, **21**, 193.

The authors are grateful to Prof. M. B. Visscher for his helpful advice and criticisms.

13431

Effects of Sulfanilamide, Sulfapyridine, Sulfathiazole and Sulfanilylguanidine upon Colon-Typhoid-Dysentery Group.

C. A. LAWRENCE AND K. D. SPRAGUE. (Introduced by O. W. Barlow.)

From the Research Laboratories of the Winthrop Chemical Co., Inc., Rensselaer, N.Y.

Recent studies of Marshall, *et al.*,[1] indicate that sulfanilylguanidine compares favorably with the *in vitro* effects of sulfanilamide and sulfapyridine upon microörganisms, particularly those of the colon-typhoid-dysentery group. Following incorporation of sulfanilylguanidine in the diet of normal mice the *in vitro* effects were largely reproduced under *in vivo* conditions in that such medication caused a distinct reduction in the usual number of coliform organisms in the stools.

Since earlier observations[2, 3, 4] indicated that sulfathiazole was equal to, or slightly superior to, sulfanilamide and to sulfapyridine in its bacteriostatic action upon organisms of the colon-typhoid-dysentery group, it appeared worth while to further study the effects of sulfanilamide, sulfapyridine and sulfanilylguanidine upon additional organisms of the group mentioned and to compare them with sulfathiazole and sodium sulfathiazole under corresponding experimental conditions.

The *in vitro* test procedures used in evaluating the several compounds were, with slight modifications, those described by Marshall.[1] Small inocula (0.2 cc) containing 50 to 750 organisms of highly diluted 18-24-hour cultures in tryptone water, were exposed to 5 cc quantities of varying drug concentrations ranging from 100 to 200 mg %. The organisms tested included: 5 strains of *E. typhosa,* 6 strains of *S. dysenteriae,* 2 strains each of *E. coli, A. aerogenes* and *Ps. aeruginosa,* and 1 strain each of *S. paratyphi, S. schottmuelleri, S. suipestifer, S. psittacosis, S. enteritidis* and *B. proteus.*

---

[1] Marshall, E. K., Jr., Bratton, A. C., White, H. J., and Litchfield, J. T., Jr., *Bull. Johns Hopkins Hosp.*, 1940, **67**, 163.

[2] Long, P. H., and Bliss, E. A., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 324.

[3] Lawrence, C. A., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 162.

[4] Libby, R. L., and Joyner, A. L., *J. Inf. Dis.*, 1940, **67**, 67.

Our observations confirm and extend the observations made by Marshall, *et al.,* in that sulfanilylguanidine corresponds in activity to sulfanilamide and is equal to or slightly inferior to sulfapyridine when tested against the organisms of the colon-typhoid-dysentery group.

In general, these investigations indicate that sulfathiazole and sodium sulfathiazole are somewhat more effective *in vitro* both as to bacteriostatic and bactericidal actions than sulfapyridine and distinctly superior to sulfanilamide and to sulfanilylguanidine against the strains tested.

A study was also made on the influence of oral medication (through incorporation in the diet) of sulfanilamide and the several derivatives on the bacterial flora of the intestinal tract of normal mice. The method and procedures followed, duplicated as closely as possible those described by Marshall, *et al.*[1] Healthy albino mice properly marked and of approximately equal weight and age were placed in coarse wire screen cages in groups of 5 animals each and fed a normal laboratory diet (Rockland mouse diet). Stool specimens were obtained directly from each mouse and emulsified in sterile broth (1.0 cc broth to each 10 mg quantity of stool). Pour plates were made in desoxycholate agar with 1 cc amounts of a 1:100 broth dilution of the heavy stool suspension. After 24 hours' and 48 hours' incubation at 37°C., the coliform colonies were counted or estimated. At least 3 control fecal specimens were taken at the same time of day on different days in order to observe the range of a normal mouse colon-bacillus count. Considerable variation was noted in the coliform population in the stools from day to day in individual mice as well as in different mice of a group on the normal diet as indicated in the tables. The magnitude of the variation noted was such that the method can be considered as of qualitative value only, if carefully controlled over a period of days.

Similar plate counts were made on stool specimens from mice 48 and 96 hours after being placed on a diet containing 1% of one of the several sulfanilamide compounds. Additional plates were made several days after the animals had been returned to a normal diet. Several of the compounds were tested alternately after intervening suitable rest periods on the same group of animals. The results of these studies are presented in Tables I, II, III.

TABLE I.

Effects of Sulfanilylguanidine and Sulfathiazole upon Bacterial Flora (*B. coli*) of Intestinal Tracts of Mice.

	Desoxycholate agar plate (*B. coli*) count Mouse No.																			
Test day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Normal diet.																				
1st	*59	H	85	H	51	10	T	H	37	H	M	71	H	27	H	M	H	H	52	H
3rd	—	T	H	H	H	50	T	T	H	T	H	H	T	28	H	T	M	0	H	H
13th	5	T	H	T	T	H	M	M	H	M	H	T	T	T	T	T	M	0	M	M
1% sulfanilylguanidine in diet on 13th day.																				
15th	8	H	50	60	H	H	16	22	15	20	M	31	H	19	65	51	M	T	T	H
17th	H	H	H	H	16	0	1	4	1	9	H	21	M	39	H	32	H	M	M	T
Normal diet begun 17th day.																				
21st	50	52	M	M	18	0	T	H	H	0	M	4	H	1	H	T	M	H	M	M
1% sulfathiazole in diet on 21st day.																				
23rd	22	18	M	H	2	2	H	4	M	0	0	20	M	4	M	55	M	H	M	T
25th	0	75	69	M	H	1	H	0	M	M	0	0	38	0	17	M	T	55	H	1
Normal diet begun 25th day.																				
36th	M	H	50	H	M	M	65	T	M	M	H	T	M	—	T	M	M	M	M	M
43rd	M	T	M	H	H	30	M	20	T	M	30	H	M	5	H	H	H	H	T	M

* Figures represent actual number of lactose-fermenting colonies in plate; H, Hundreds; T, Thousands; M, Innumerable; 0, No lactose-fermenting colonies observed; —, No test made.

TABLE II.

Effects of Several Sulfonamide Compounds upon Bacterial Flora (*B. coli*) of Intestinal Tracts of Mice.

	Desoxycholate agar plate (*B. coli*) count. Mouse No.																			
Test day	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Normal diet.																				
1	*H	M	H	H	H	M	H	T	M	H	H	H	3	T	H	H	50	36	M	H
3	H	H	50	H	H	M	M	H	M	M	H	18	H	M	44	M	4	M	M	H
7	H	H	M	M	T	M	M	30	M	T	H	M	70	T	M	T	0	50	T	M
Drug diet begun 7th day.																				
	1% Sulfanilylguanidine										1% Sulfathiazole									
9	T	50	0	6	2	T	H	20	H	3	15	H	0	H	H	2	0	0	2	6
11	T	H	1	H	50	H	H	25	11	50	15	H	35	H	H	60	0	10	3	10
Normal diet begun 11th day.																				
13	M	50	70	M	M	T	H	M	M	50	32	M	M	H	20	T	H	2	M	H
15	T	H	20	M	T	M	46	30	M	H	50	M	H	H	M	50	M	1	T	H
16	T	M	10	T	M	M	18	35	M	15	H	M	0	H	M	20	M	15	H	H
Drug diet begun 16th day.																				
	1% Sulfapyridine										1% Na Sulfathiazole									
18	H	T	2	10	M	H	25	30	55	30	4	20	2	0	6	8	0	13	19	5
20	H	M	3	28	65	H	H	23	T	20	40	5	0	0	20	8	1	6	60	4
Normal diet begun 20th day.																				
23	H	4	H	H	T	H	12	67	H	31	51	46	24	M	M	20	16	21	44	52
27	T	M	T	H	M	H	0	0	T	M	H	6	T	T	T	13	H	0	2	T
28	H	M	65	90	H	T	T	H	T	H	H	50	H	M	M	7	90	0	T	H

*See legend under Table I.

TABLE III.
Effects of Sulfanilamide and Sulfathiazole upon Bacterial Flora (*B. coli*) of Intestinal Tracts of Mice.

	Desoxycholate agar plate (*B. coli*) count Mouse No.									
Test day	1	2	3	4	5	6	7	8	9	10
	Normal diet.									
1	*H	H	T	M	M	M	H	M	H	M
3	M	M	H	M	T	M	T	M	M	M
7	50	M	T	T	T	H	T	H	H	T
	1% Sulfanilamide in diet on 7th day.									
9	M	M	H	T	H	H	M	T	H	H
11	M	M	M	T	M	H	M	H	24	H
	Normal diet begun 11th day.									
13	T	M	T	T	M	H	M	H	M	51
15	M	M	M	M	M	T	M	M	M	H
16	M	M	M	M	M	H	M	M	H	H
	1% Sulfathiazole in diet on 16th day.									
18	90	T	70	T	75	30	0	1	10	T
20	50	0	22	T	21	18	19	14	20	dead
	Normal diet begun 20th day.									
23	T	65	T	T	M	35	H	H	34	
27	T	50	M	T	13	H	40	60	H	
28	T	T	H	M	H	60	21	3	5	

*See legend under Table I.

In summarizing the data in the tables, the findings of Marshall were confirmed in that the normal fecal counts of coliform organisms of many of the animals were reduced following dietary medication with sulfanilylguanidine.

Upon extending the feeding experiments to include sulfapyridine, sulfathiazole and sodium sulfathiazole, a similar reduction in the *B. coli* population in the fecal excreta was obtained with all compounds. The animals medicated with sulfanilamide, on the other hand, failed to show a decrease in the *B. coli* count of their stool specimens (Table III). However, substitution of a sulfathiazole, sodium sulfathiazole, sulfapyridine or sulfanilylguanidine for the "sulfanilamide diet" invariably caused a significant reduction in the number of coliform organisms.

The wide variations noted in the *B. coli* counts of fecal specimens of mice on normal diets and the corresponding erratic, although depressed, counts noted after medication with all compounds with the exception of sulfanilamide suggest that this test method for chemotherapeutic compounds is of qualitative value only. Thus, results are influenced primarily by the relative absorbability of the medicaments and secondarily by the dietary drug concentration or dosage fed. Drug action *per se* might, therefore, play only a minor rôle.

Conclusions. *In vitro* studies on several sulfonamide derivatives indicate sulfathiazole and sodium sulfathiazole to be somewhat more effective than sulfapyridine and distinctly superior to sulfanilamide and to sulfanilylguanidine against the colon-typhoid-dysentery group.

Feeding the several compounds in the diet to mice, caused, with a single exception, a significant reduction of the number of coliform organisms normally present in the intestinal excreta. Sulfanilamide, under the conditions of this test, proved to be ineffective in lowering the fecal *B. coli* count of the animals.

13432

Mechanism of the Effect of Epinephrine on Bioluminescence of the Firefly.

G. A. EMERSON AND MARJORIE JEAN EMERSON.

From the Department of Pharmacology, West Virginia University School of Medicine, Morgantown.

There is general agreement[1-4] that flashing of fireflies is regulated by innervated tracheal end-cells which normally occlude tracheoles leading to the light organ. The effect of epinephrine may be interpreted[1] as dilatation of the occluding musculature to permit constant access of O_2 to photogenic areas with consequent prolonged luminescence. Use was made of this action to demonstrate[5] that tensions of ether vapor above the minimal lethal tension will extinguish the constant bright glow of fireflies treated with epinephrine although ether vapor itself causes a constant glow in untreated fireflies exposed to sublethal tensions. Enough specimens were captured from a swarm of the lampyrids, *Photuris pennsylvanica*, during the summer of 1941, at Suncrest, W. Va., to permit the present limited further study.

A recent note by Chakravorty and Ballentine[6] mentions the effect of hydrosulfide on luciferase-oxidized luciferin. Accordingly, 0.01-0.02 cc of 1% freshly prepared NaHS was injected intraäbdominally in 10 fireflies. A moderately bright glow developed immediately and persisted for several minutes, gradually diminishing. Since most available solutions of epinephrine contain 0.1-0.2% $NaHSO_3$, a commercial grade of bisulfite was injected in concentrations of 0.1-1%. With the stronger concentration, a moderately bright luminescence appeared for a short time in 8/10 fireflies and a bright glow in 2. With the weaker solution, a very weak glow developed in 10/10, lasting about 5 minutes. Pure 0.1% epinephrine HCl, prepared from Eastman epinephrine, caused the same constant bright glow lasting several hours in 30/30 fireflies as was noted with commercial 10^{-3} epinephrine. Contaminants in commercial epinephrine solutions are not responsible for the prolonged bright luminescence of fireflies treated with these solutions.

---

[1] Creighton, W. S., *Science*, 1926, **63**, 600.

[2] Gerretsen, F. C., *Biol. Zentralbl.*, 1922, **42**, 1.

[3] Harvey, E. N., *Physiol. Rev.*, 1924, **4**, 639.

[4] Snell, P. A., *Science*, 1931, **73**, 372; *J. Cell. Comp. Physiol.*, 1932, **1**, 37.

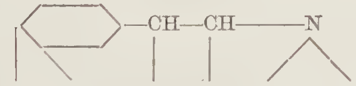
[5] Emerson, G. A., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 36.

[6] Chakravorty, P. N., and Ballentine, R., *J. Am. Chem. Soc.*, 1941, **63**, 2030.

Further corroborative evidence is desirable in support of Creighton's thesis[1] that epinephrine acts through physical improvement of the O_2 supply, before the alternate mechanism of a more intimate interference as a reducing agent may be dismissed. The effect of epinephrine on photogenic glandular structures of the singing-fish, *Porichthys notatus,* is believed[7] to be directly on these light organs rather than through a nervous mechanism. Epinephrine decreases bacterial luminescence,[8] however, and Shoup and Kimler[9] have shown that 2,4-dinitrophenol also does not stimulate luminescence.

The following observations were made using an average of 10 ♂ fireflies for each agent. Conclusions are drawn from mean results of intraäbdominal injection of 0.01-0.02 cc of 1% solutions of the pure agents in 0.5% NaCl, except as noted. Hydrochlorides or sul-

TABLE I.
Effects of Certain Sympathicomimetic Amines on Bioluminescence in Fireflies.

Agent	Structure						Duration and intensity of induced glow	
							Duration, min.	Intensity
Phenethylamine	H	H	H	H	H	H	5	bright
Phenethanolamine	H	H	OH	H	H	H	15	”
							>120	moderate
Benzedrine	H	H	H	CH_3	H	H	5	”
Propadrine	H	H	OH	CH_3	H	H	10	”
l-Ephedrine	H	H	OH	CH_3	H	CH_3	15	”
							> 60	weak
d-Ψ-Ephedrine	H	H	OH	CH_3	H	CH_3	> 15	very weak
Neosynephrine, 0.25%	H	OH	OH	H	H	CH_3	15	bright
Tyramine	OH	H	H	H	H	H	15	”
							>360	weak
Hordenine	OH	H	H	H	CH_3	CH_3	120	bright
							>600	weak to moderate
Epinephrine, 0.1%	OH	OH	OH	H	H	CH_3	>360	bright

---

[7] Greene, C. W., and Greene, H. H., *Am. J. Physiol.*, 1924, **70**, 500.

[8] Taylor, G. W., *J. Cell. Comp. Physiol.*, 1932, **1**, 297.

[9] Shoup, C. S., and Kimler, A., *J. Cell. Comp. Physiol.*, 1934, **5**, 269.

fates of the bases were used and other agents were employed as noted, either as free acid or Na salt. Table I presents data for the sympathicomimetic amines studied.

Neither freshly prepared solutions of l-dihydroxyphenylalanine nor the same after 24 hours of eremacausis have any effect. Homogentisic and mandelic acids, and mescaline are also without effect. Resorcinol, pyrocatechol and pyrogallol produce a slow, spotty, very weak glow. A 1% suspension of tyrosine induces a weak glow in 3/10 fireflies and greatly stimulates flashing. Tyrosine partly dissolved in a large excess of $NaHCO_3$ causes a bright spotty glow for about an hour but a similar effect is produced by strong solutions of $NaHCO_3$ alone, presumably due to osmotic phenomena.

Yohimbine, ergotamine tartrate, piperidino-methylbenzodioxane and diethylamino-methylbenzodioxane have no effect alone but reduce the persistence of the bright glow after treatment with epinephrine to about 1 hour. Part of this action may be through adrenolytic effect but control injections of 0.5% NaCl also have an appreciable antagonistic effect, through dilution of the epinephrine. Cocaine has no effect alone and does not appear to potentiate the effect of 10^{-7} epinephrine, which produces a faint, spotty glow alone.

Atropine, hyoscyamine, homatropine, hyoscine, and trasentin either have no effect or produce a very weak glow. While none has any appreciable immediate effect, a persistent weak glow develops 30 minutes after injection of trasentin and less uniformly after atropine or hyoscyamine. The same latent effect is apparent several hours after the immediate effect of some of the weaker sympathicomimetic amines and may be related simply to general toxic action. A 1% emulsion of benzylbenzoate in 3% ethanol causes a very weak glow in about half the treated fireflies. Octin also causes a very weak glow. Papaverine produces a bright glow for 15 minutes followed by a persistent weak glow lasting more than 15 minutes.

Nicotine causes a weak to moderate glow in half the treated fireflies but is almost immediately lethal. Similar irregular effects on luminescence are produced with toxic concentrations of other agents which produce no effect in sublethal amounts, such as N_2O and ethylene,[10] and these can be considered as a common phenomenon postmortem. Fireflies exhibit an amazing tolerance towards lethal effects of other agents studied.

$NaNO_2$ causes a bright glow for 10 minutes. $BaCl_2$ produces a weak, spotty glow with much stimulation of flashing for 10 minutes. A commercial preparation of kallikrein, 7U/cc in 60% glycerol, produces a bright glow becoming spotty in 1 hour but glycerol alone also produces this effect. 60% glucose causes a moderately bright glow for 30 minutes. Since 40% NaCl will produce the same intensity and duration of glow, it is more probable that glucose and glycerol act through osmotic effects than as reducing substances. The action of $NaHCO_3$ referred to above is also probably osmotic since weak buffer solutions of pH 2-13 have no effect. A weak, spotty glow follows injection of solutions of pH 1.

---

[10] Emerson, G. A., *Anesthesia and Analgesia*, 1936, **15**, 134.

Injection of active amounts of O_2 dissolved in any of the solutions is improbable since 0.5% NaCl alone has no effect. Injection of air sometimes causes a weak spotty glow lasting less than 1 minute. Injection of O_2 causes a bright glow for 30 seconds followed by a very weak glow for 5 minutes. H_2O_2 produces a bright glow lasting less than 5 minutes and ammonium o-iodoxybenzoate has no effect but a momentary weak glow. These solutions contain more rapidly available O_2 than any of the other test solutions, and peroxide has a stimulant effect[11] on bacterial luminescence.

Effects of sympathicomimetic amines on luminescence of fireflies are analogous to pressor effects of these agents in higher forms of life, and the influence of phenolic and alcoholic hydroxyl groups and of substitution of the amino group is much the same in both instances. The extraordinary activity of epinephrine is related to the molecule as a whole and not to the influence of any single chemical group. Lack of significant effect of phenolic compounds not active as sympathicomimetics emphasizes the unimportance of catechol or similar groups alone. It is unlikely that the effect of epinephrine on fireflies is dependent on participation of the catechol group in the luminescence reaction. Whether the effect is directly on muscular tissue, on a receptive mechanism, or directly or remotely on nerves innervating the end-cells is not definable without a quantitative study of pharmacologic effects of antagonists and potentiating agents beyond the scope of the present study.

Summary. The action of epinephrine is not mediated through direct chemical stimulation of the luminescence reaction. Consideration of relative potencies of its congeners in evoking constant luminescence in fireflies suggests that the site of action is at the tracheal end-cells and that no nicotine-like effect is involved.

---

[11] Nakamura, H., *Acta Phytochim.* (Japan), 1939, **11**, 159.

13433

Thiamin in Sweat.

Leo L. Hardt and Eugene U. Still.

From the Department of Medicine, Loyola Medical School, and Biochemical Research Laboratories, Chicago, Ill.

It is now well known that thiamin requirements are increased with diuresis, exercise, exposure to high temperature, and the administration of thyroid. For this reason we thought it desirable to study the possible loss of thiamin in the sweat as part of a problem on the causation of fatigue. Several authors have published papers on the excretion of various crystalloids in the sweat. The excretion of ascorbic acid was first noted by one of us in 1934 and reported in 1935 at a meeting of the Chicago Tuberculosis Society. Later quantitative estimations were made of ascorbic acid in the sweat by Cornbleet, Klein, and Pace;[1] also by Wright and MacLenathen.[2] The weight losses as a result of sweating have interested many investigators. Snapper and Grunbaum[3] report a loss of 1 to 2 kg in 2 hours of football. Krestownikoff[4] found a sweat loss of 350 to 1530 g per hour in heavy work under ordinary temperatures. Bock and Dill[5] in their research at Boulder City found that physical activities at high temperatures produced great losses of chlorides as well as water from the body—as much as 20 lb of sweat in a 7 hour period. Caspari and Schilling[6] pointed out that subjects living in the tropics may eliminate as much as 3 to 4 kg of sweat a day even when they do not feel any sensible perspiration.

Our first studies were undertaken in a room with a temperature of 160°F, on 4 normal male subjects, one of whom had taken about 50 mg of thiamin one hour before entering the hot room. A pooled sample of about 25 cc of sweat was taken from these 4 individuals; when tested for thiamin, it contained approximately 1.5μg per cc.

Following this observation, experiments were performed with 24 football players from Concordia College in River Forest, Illinois. The men were divided into 4 groups of 6 each. The men in 2 of the groups were given 50 mg of thiamin chloride and 750 mg of ascorbic acid by mouth previous to football practice (fortified group). The men in the other 2 groups were given no additional vitamins (unfortified group). Each subject was weighed, a complete specimen of urine was obtained, and 10 cc of blood were withdrawn before the practice. At the end of one hour of scrimmage Groups I (unfortified) and II (fortified) returned to a warm room, about 80°F, were given 2 cups of warm tea, immediately stripped to the waist and the sweat collected from the chest anteriorly and posteriorly. Groups III (unfortified) and IV (fortified) remained at active scrimmage 30 minutes longer. Another complete specimen of urine was obtained, together with 10 cc of blood, and they were weighed again. The sweat was pooled for each group to insure a sufficient quantity for duplicate determinations.

---

[1] Cornbleet, T., Klein, R. I., and Pace, E. R., *Arch. Dermat.*, 1936, **34**, 253.

[2] Wright, I. S., and MacLenathen, E., *J. Lab. and Clin. Med.*, 1939, **24**, 804.

[3] Snapper, L., and Grünbaum, A., *Bioch. Z.*, 1929, **206**, 319.

[4] Krestownikoff, A. N., *Z. ges. exp. Med.*, 1930, **74**, 200.

[5] Bock, A. V., and Dill, D. B., *New Eng. J. Med.*, Oct. 31, 1933.

[6] Caspari, W., and Schilling, C., *Z. Infektkr. Hyg.*, 1921, **91**, 57.

The analyses for thiamin were made according to a technic based upon the fluorometric determination of thiochrome. Ascorbic acid was titrated with indophenol and read directly and by means of an electro-photometer.

From our findings thus far, (Table I), certain inferences may be drawn.

1. In Group I (unfortified) the concentration of thiamin in the sweat is double that of the urine after 1 hour of exercise. In Group III (unfortified) that had ½ hour additional exercise the concentrations in both urine and sweat were slightly lowered, but the sweat concentration was still double that of the urine.

2. In Group II (fortified) after 1 hour the sweat concentration was 71 times the concentration of the urine. In Group IV (fortified) that had ½ hour of additional exercise the concentration of the thiamin in the sweat was only 8.5 times that of the urine.

3. Since the concentration of thiamin in the sweat in Group III (unfortified) which exercised ½ hour longer is less than in Group I (unfortified), one may conservatively estimate the total loss in sweat on the basis of Group III. Since the average weight loss in this group was 1.13 kg and the concentration was 82.5 micrograms per liter, the total loss would be 93.2 μg in 1½ hours of sweating. This may be compared with the urine sample collected in the same period which contained 4.6 μg in 107 cc. In 170 cc of urine collected before exercise there were 7.6 μg excreted in this group. Hence the excretion of thiamin in sweat does not seem to be compensated for by appreciable diminution in the urine.

4. In the fortified groups the concentration of thiamin in the sweat in Group II was 10 times the concentration in the sweat in Group IV, showing a great pouring out of thiamin early in the sweat period. No estimation of total loss is therefore possible by this method.

TABLE I.
The Average Results for Each Group of Subjects.

		Urine 1			Urine 2		
Group	Vitamins given	Vol., cc	Ascorbic acid, mg/L	thiamin, μg/L	Vol., cc	Ascorbic acid, mg/L	thiamin, μg/L
I	0	157	21	45.7	117	21.6	41.6
II	C, B_1	164	39.5	46	107	93.9	63.6
III	0	170	15.2	45	107	26.7	41.6
IV	C, B_1	200	9.07	42	141	21.8	52.3

		Blood 1		Blood 2		Sweat		
Group	Vitamins given	Ascorbic acid, mg/L	thiamin, μg/L	Ascorbic acid, mg/L	thiamin, μg/L	Ascorbic acid, mg/L	thiamin, μg/L	wt loss, kg
I	0	0.67	7.78	0.56	7.99	2.01	90	1.25
II	C, B_1	0.84	7.41	0.78	14.15	3.59	4540	1.15
III	0	0.53	7.60	0.61	11.40	1.76	82.5	1.13
IV	C, B_1	0.67	7.71	0.62	15.40	4.66	445	1.16

Group I and II (unfortified) were not given vitamin C and B before exercise.

Group II and IV (fortified) were given 750 mg of ascorbic acid and 50 mg of thiamin before exercise.

Group III and IV exercised ½ hour longer than Group I and II.

5. The thiamin in the blood tended to rise with sweating, especially in the fortified groups where the concentration was practically doubled.

6. The average concentration of thiamin in the sample of urine and blood collected from the subjects studied would indicate a probable low normal intake. There was a slight rise in the fortified groups and a slight decrease in the unfortified groups, indicating body loss mostly through sweat.

Ascorbic acid studies indicate that the subjects are on a low normal ascorbic acid diet. There was a slight elevation in the urine after sweating, especially in the fortified groups where the concentration was practically double. In the blood the ascorbic acid remained practically at a constant level with the sweating, and did not change after exercise or after fortifying the subjects with additional amounts by mouth. In the fortified groups the concentration of ascorbic acid in the sweat was slightly higher in the group that exercised 1½ hours than in the 1-hour group in contrast to thiamin. This suggests that the peak of ascorbic acid excretion may be reached later than thiamin or may be due to slower absorption of ascorbic acid. In the unfortified group that had exercised 1½ hours the concentration is lower than the group that exercised 1 hour, suggesting that the peak of excretion had passed. The concentration of ascorbic acid in the urine is roughly 5 to 10 times that in the sweat, but the actual amount of ascorbic acid excreted during the exercise is roughly the same.

Thiamin is normally excreted in appreciable amounts in the sweat. Since a man may readily sweat (moderate temperature and work) 2 to 3 kg daily without visible perspiration, he may thus lose in the sweat from 5 to 15% of ingested thiamin. In a like manner, a person undergoing hard work at elevated temperatures may lose enough thiamin to cause serious physiological consequences. Our work indicates that the increased excretion of thiamin in sweating is not compensated for by a diminution of the urinary thiamin.

The relationships of the above inference to the problem of work and fatigue are obvious. Clinical studies concerning certain cases of apparently abnormal vitamin requirements are also indicated.

The authors wish to express their appreciation to Drs. F. C. Bing and William Bradley for their interest and helpful suggestions in this work.

13434

Experimental Transmission of Saint Louis Encephalitis to White Swiss Mice by *Dermacentor variabilis.*

Russell J. Blattner and Florence M. Heys. (Introduced by Jean V. Cooke.)

From the Department of Pediatrics, Washington University, and the St. Louis Children's Hospital.

In studies of Russian encephalitis various investigators[1-6] have reported observations which indicate that *Ixodes* ticks play an important part in the epidemiology of the disease. Laboratory experiments conducted by some[4, 6] of these workers demonstrated that members of the genus, *Ixodes,* are capable of transmitting the virus to susceptible animals during larval, nymphal, and adult stages of the life cycle. There was considerable evidence also that the infected

---

[1] Editorial, *J. A. M. A.*, 1941, **117**, 1361.

[2] Smorodintseff, A. A., *Arch. f. Ges. Virusforsch.*, 1940, **1**, 468, quoted by 1.

[3] Rijov, N. V., and Skrynnik, A. N., *Report of the All-Union Congress of Microbiology,* 1939 (Jan. 25-31), quoted by 1.

[4] Pavlovsky, E. N., *Report of the All-Union Congress of Microbiology,* 1939 (Jan. 25-31), quoted by 1.

[5] Chumakov, M. P., and Seitenok, N. A., *Science,* 1940, **92**, 263.

[6] Shubladse, A. K., and Serdinkova, G. V., *Arch. Soc. Biol.,* 1939, **56**, No. 2.

female tick transmits the virus to her offspring by inclusion in the egg. Ticks collected in nature in endemic areas were found to harbor the virus of Russian encephalitis. More recently Syverton and Berry[7] reported the transmission of equine encephalomyelitis to ground squirrels by the tick, *Dermacentor andersoni.*

These observations raised the question of the possible rôle of the tick in some phase of the epidemiology of Saint Louis encephalitis. Ticks, collected from the environs of Saint Louis and identified as *Dermacentor variabilis,* were maintained as a stock colony. Females of the colony, fed by engorging on normal dogs, lay eggs within 4 days after engorgement, and larvae emerge 15 days later.

In the present experiments adult white Swiss mice were inoculated with St. Louis encephalitis virus, each receiving 0.03 cc 10^{-1} intracerebrally and 0.1 cc 10^{-1} intraperitoneally. Thirty to 40 normal larval ticks hatched in the laboratory were allowed to come into contact with each of 4 such inoculated mice in separate glass jars. Attachment occurred immediately, favorite sites being the ears and snout. Inoculated mice were unsuccessful in dislodging the larval ticks once they had attached. After an interval of 2 days one or 2 normal Swiss mice, 2 weeks of age, were placed in each glass jar with an infected mouse and larvae. The young mice nestled against the infected adults (males), but made no apparent effort to remove the larvae. The attached larval ticks became engorged with blood, increasing in size at about the expected rate. Within 4 days the inoculated adult mice died in typical encephalitic convulsions. The larval ticks promptly left the dying adults, and a number attached themselves to the young mice. Ten days after the death of the inoculated adult mice, one of the 6 young mice in this series was found dead; another was found in convulsions. Twenty-four hours later 2 more young mice developed convulsions. In each instance the virus was recovered from the brain by passage to normal Swiss adults and to fertile eggs, and was identified as the Saint Louis type by mouse and egg protection tests[8] with known hyperimmune rabbit serum. The virus was maintained readily by serial passage in mice and eggs. Autopsies on all dead or dying mice revealed no evidence of bacterial invasion, and cultures of brain and lung tissue were sterile in every case. The remaining 2 of the 6 young mice showed vague symptoms but survived.

These results are in accord with the suggestive findings of a preliminary experiment similar to the foregoing in which 5 of 8 two-weeks-old mice died during the night, 7 days after first contact with infected larval ticks. In this preliminary series no effort was made to recover the virus.

---

[7] Syverton, J. T., and Berry, G. P., *J. Exp. Med.*, 1941, **73**, 507.

[8] Blattner, R. J., and Cooke, J. V., *J. Inf. Dis.*, in press.

In an attempt to demonstrate the presence of the virus in the arthropod vector, larval ticks which had engorged on mice infected by intracerebral and intraperitoneal inoculation were removed carefully from dying mice and ground in broth in a micromortar. The supernatant fluid was injected intraperitoneally into 2-weeks-old mice. In a group of 6 such mice inoculated with ground tick suspension, 3 developed convulsions within 7 to 8 days, and one was found dead on the eighth day following inoculation. The brains of these 4 when passed to normal adults produced typical convulsions and death in 2½ to 3 days.

While it is generally accepted that Saint Louis encephalitis is not contracted by contact exposure, such a possibility exists, especially since Smith,[9] and also King,[10] have shown that young mice are much more susceptible to small amounts of virus. For this reason the following experiment was performed as control. Two-weeks-old mice were placed with infected adults and allowed to remain during the course of illness until several hours after the latter had died. No symptoms of encephalitis developed in these young mice.

In an effort to check the possibility of infection through the gastrointestinal tract by ingestion of infected larval ticks, young mice (*i. e.*, 2 weeks of age) were fed food grossly contaminated by mixture with infected brain tissue. All such animals failed to develop encephalitis, which is in keeping with the observations of Brodie,[11] who also was unable to infect normal mice by ingestion of food grossly contaminated by Saint Louis encephalitis virus. Harford, Sulkin, and Bronfenbrenner,[12] while in general reaching the same conclusion observed, however, that 2 of 7 normal adult mice did contract encephalitis by eating moribund new-born mice previously infected by the intracerebral route. The authors attributed these results to the large quantity of virus ingested. The further possibility of infection by the intranasal route was also considered, since King[10] concluded that this route is the most effective peripheral means of inoculation. Specific experiments are being conducted to investigate this possibility, although nasal infection from ingestion of ticks seems unlikely.

---

[9] Smith, M. G., *J. Exp. Med.*, in press.

[10] King, L. S., *J. Exp. Med.*, 1940, **72**, 573.

[11] Brodie, M., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 1647.

[12] Harford, C. G., Sulkin, S. E., and Bronfenbrenner, J. J., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 332.

Further evidence that the tick can transmit Saint Louis encephalitis was obtained in experiments with full-grown ticks and adult mice, since such adult mice upon which infected full-grown ticks had fed developed considerable resistance to intracerebral inoculation of virus. Full-grown ticks, which had engorged for a period of 5 to 7 days on infected adult mice, were transferred to normal adults and continued feeding for a like period. None of the latter developed convulsions, and all survived, although they seemed ill for several days showing lethargy, ruffled fur, and lack of interest in food. Later these convalescent mice were tested by intracerebral injection of Saint Louis encephalitis virus which was sufficient to kill, without exception, all normal controls in 3 to 5 days. Twelve of 17 mice so tested have survived, showing an apparently increased resistance to intracerebral inoculation. Here the possibility of infection by ingestion or by the intranasal route was obviated since adult ticks were protected during attachment by a specially contrived jacket having a small glass perforated chamber into which the tick may expand during engorgement.

Summary. 1. The virus of Saint Louis encephalitis was recovered from the bodies of engorged larval ticks which had fed on infected adult Swiss mice. 2. Under experimental conditions the Saint Louis encephalitis virus was transmitted to young Swiss mice by larval ticks. 3. Normal adult mice upon which infected, full-grown ticks had fed developed considerable resistance to intracerebral inoculation of virus.

13435

Vitamin A Concentration in Rat Liver During Recovery from CCl_4 Cirrhosis.*

CHARLES HAIG AND JOSEPH POST.

From the Research Service, First Medical Division, Welfare Hospital, and the Department of Medicine, College of Physicians and Surgeons, Columbia University, New York City.

It has been found that the liver contains approximately 95% of the body stores of vitamin A,[1] and is the site of production of the vitamin from its carotenoid precursors.[2] The vitamin A content of this organ is therefore a valuable index of vitamin A metabolism. Low levels of the vitamin in the blood[3-6] as well as in the liver[6-10] have been reported in human cirrhosis of the liver. Since the liver vitamin A may be decreased in amount by reduced vitamin A intake[11, 12] or by faulty gastrointestinal absorption,[9, 13] particularly as affected by the presence of jaundice,[14] studies were conducted in the rat in which an attempt was made to control these factors.

---

* Reported in preliminary form to the American Society of Zoölogists, *Anat. Rec.*, 1941, **81**, Suppl., 124.

[1] Moore, T., *Biochem. J., London*, 1931, **25**, 275.

The data reported were obtained from rats in the recovery stage of carbon tetrachloride cirrhosis. At this stage the liver cells appear normal and the degree of fibrosis is moderate.[15] During the period of study the animals were free of jaundice and of diarrhea.

The effect of feeding different quantities of brewers' yeast on the concentration of vitamin A in cirrhotic livers was also examined.

Methods. Twenty-five 10-week-old, male rats, of Sherman stock, were employed. Nineteen of these were injected subcutaneously, twice weekly, with a 50% solution of CCl_4 in mineral oil, in a dosage of 0.05 ml per 100 g of body weight. The 6 animals receiving no CCl_4 served as controls.

The diet employed consisted of sucrose 69%, casein* 18%, butter 8%, Hawk-Oser salt mixture[16] 4%, supplemented by 0.3-2% of brewers' yeast.† The animals were fed *ad lib.* The CCl_4 injections were administered for 8 weeks, a period found sufficient to produce severe cirrhosis,[15] after which time the injections were discontinued.

---

[2] Wilson, H. E. C., Ahmad, B., and Mazumdar, B. N., *Indian J. Med. Res.*, 1937, **25**, 85.

[3] Lasch, F., *Klin. Wochenschr.*, 1938, **17**, 1107.

[4] Lindqvist, T., *Acta med. Scand.*, 1938, **98**, Suppl. 97, pp. xii + 262 + 52.

[5] Rubegni, R., *Policlinico, sez. med.*, Rome, 1939, **46**, 565.

[6] Haig, C., and Patek, A. J., Jr., to be published.

[7] Moore, T., *Biochem. J.*, London, 1937, **31**, 155.

[8] Breusch, F., and Scalabrino, R., *Z. ges. exp. Med.*, 1934, **94**, 569.

[9] Woo, T. T., and Chu, F. T., *Chinese J. Physiol.*, 1940, **15**, 83.

[10] Ralli, E. P., Papper, E., Paley, K., and Bauman, E., *Arch. Int. Med.*, 1941, **68**, 102.

[11] McCoord, A. B., and Luce-Clausen, E. M., *J. Nut.*, 1934, **7**, 557.

[12] Lewis, J. M., Bodansky, O., Falk, K. G., and McGuire, G., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 248.

[13] Crimm, P. D., and Short, D. M., *Ann. Int. Med.*, 1939, **13**, 61.

[14] Breese, B. B., and McCoord, A. B., *J. Ped.*, 1940, **16**, 139.

[15] Post, J., Earle, D. P., Jr., Patek, A. J., Jr., and Victor, J., *Am. J. Path.*, in press.

* Grade 20, Casein Company of America.

[16] Hawk, P. B., and Oser, B. L., *Science,* 1931, **74**, 369.

† Harris Medicinal Brewers' Yeast, Tuckahoe, N.Y.

The 19 presumably cirrhotic rats[15] and the 6 normal, uninjected rats were then grouped as follows:

Group A: 10 "cirrhotic" rats received the basal ration supplemented by an average of 1% of brewers' yeast.

Group B: 9 "cirrhotic" rats received the basal ration supplemented by an average of 7% of brewers' yeast.

Group C: 6 normal rats received the basal ration supplemented by an average of 1% of brewers' yeast.

The source of vitamin A in the basal diet was butter. It was estimated[17] that this supplied 186-230 international units of vitamin A per kilo of body weight per day. This amount is 10 times the minimal requirement of vitamin A for growth as determined by Goss and Guilbert.[18] All diets were made at the same time and kept under identical environmental conditions.

Group A animals were fed *ad lib.* (12.5 g/day). Animals of group B were fed the same amount of food (12.9 g/day) as those of group A consumed. Group C animals, although fed *ad lib.*, consumed the same amount of food (13.3 g/day) as those of groups A and B. Thus the 3 groups of animals were maintained under isocaloric conditions and their vitamin A intake was the same.

The animals were kept on these rations for an additional 3 months, *during which time no more CCl_4 was administered.* All animals were then sacrificed and the body weight, liver weight, degree of hepatic fibrosis,[15] and amount of vitamin A per gram of liver tissue were determined.

The method employed for the preparation of vitamin A extracts of the livers was similar to that described by McCoord and Luce-Clausen.[11] Sampling errors were minimized by employing pooled samples taken from at least 6 different loci of each liver. The vitamin A contents of the liver extracts so prepared were determined by the method described elsewhere[19] for determining the vitamin A content of plasma extracts. The results were expressed in international units of vitamin A per gram of fresh liver tissue.

Results. In Table I are presented the body weights, liver weights, and concentrations of vitamin A in the livers of the 3 groups of animals under study. The average amount of vitamin A per gram of liver of the rats in the recovery stage of CCl_4 cirrhosis (groups A and B) is seen to be less than half that of the normal animals (group

---

[17] Daniel, E. P., and Munsell, H. E., *U. S. Dept. Agric. Misc. Publ.*, No. 275, June, 1937.

[18] Goss, J., and Guilbert, H. P., *J. Nut.*, 1939, **18**, 169.

[19] Haig, C., and Patek, A. J., Jr., to be published.

TABLE I.
Body and Liver Weights in Grams, and International Units of Vitamin A per Gram of Rat Liver.

Group A (CCl_4, 1% yeast)				Group B (CCl_4, 7% yeast)				Group C (No CCl_4, 1% yeast)			
Rat No.	Body wt	Liver wt	Vit. A/g liver	Rat No.	Body wt	Liver wt	Vit. A/g liver	Rat No.	Body wt	Liver wt	Vit. A/g liver
10	156	3.7	**5.6**	3	208	5.8	**12.0**	53	172	12.7	**27.0**
16	205	6.2	**12.6**	7	278	8.5	**21.7**	54	250	8.1	**25.9**
21	168	4.6	**5.7**	9	304	9.0	**27.6**	56	211	5.3	**12.9**
22	167	5.5	**16.2**	17	286	9.5	**7.1**	57	216	5.7	**43.7**
32	169	8.1	**7.1**	25	364	9.5	**8.5**	59	226	5.5	**27.5**
33	167	5.0	**30.7**	30	318	9.0	**3.9**	60	254	6.8	**24.4**
35	181	5.5	**9.2**	42	317	10.5	**0.0**				
41	226	7.0	**22.5**	51	250	5.5	**15.4**				
43	202	9.0	**5.0**	52	265	6.5	**22.6**				
45	237	7.5	**4.7**								
Mean			**11.9**				**13.2**				**26.9**
Standard deviation			**8.3**				**8.8**				**9.0**

C). Despite the high degree of dispersion of the vitamin A measurements, the observed differences between the mean values for the CCl_4 injected rats and the uninjected rats were found to have statistical significance.

Since changes in liver size accompanying the reduction in vitamin A concentration would alter the interpretation of these findings, it is pertinent that the average ratio of body weight to liver weight was found to be the same for the two groups of animals, *viz.,* 33.4 for the control and 33.6 for the injected rats. Hence it is probable that the CCl_4 injections had no effect upon the size of the livers of the injected animals. Correlatively, it was found that for the injected rats the average ratio of the total vitamin A contents of the livers divided by the body weights was less than half that for the control rats. Hence the reduced concentration of vitamin A in the livers of the CCl_4 injected animals involved a proportionate reduction in the quantity of vitamin A available to the body from the liver, as well as in the total quantity of vitamin A stored in the liver.

No apparent correlation was found between the vitamin A concentration, the degree of fibrosis observed in microscopic sections of the individual livers,[15] and the yeast intake of the animals. This finding with regard to yeast intake confirms in part the observations of Dann and Moore.[20]

The data of Greaves and Schmidt[21] suggest that the administration of phosphorous for 6 days causes some interference with the conversion of carotene to vitamin A in the liver. These authors also state that the administration of $CHCl_3$, benzene, or CCl_4 (for an unspecified number of days) to vitamin A-deficient rats, in the "dosage found possible to administer," had no demonstrable effect on the carotene conversion when this was determined by the vaginal smear method.

---

[20] Dann, W. J., and Moore, T., *Biochem. J.*, London, 1931, **25**, 914.

[21] Greaves, J. D., and Schmidt, C. L. A., *Am. J. Physiol.*, 1935, **111**, 503.

Studies by Wendt and König[22] and Cox[23] were concerned with comparisons between the vitamin A content of different portions of the same diseased liver, but offer no data comparing these values with those found in normal livers. Our analysis compared the vitamin A content of diseased and normal livers. Other studies[22, 24] of the influence of liver damage on storage of vitamin A in the liver, unlike the present experiments, were concerned with poisoning of the liver for only a few days. Moreover, in none of these experiments was CCl_4 employed as the poisoning agent.

It is important to emphasize that the CCl_4-injected rats of the present study had remained untreated for 3 months before the vitamin A analyses were made. At this stage the degree of liver fibrosis was minimal and the liver cells appeared normal. Despite these facts the livers of the injected animals contained less than half the amount of vitamin A found in the livers of the normal controls.

Summary. Nineteen rats were rendered severely cirrhotic by the bi-weekly administration of carbon tetrachloride over a period of 2 months. Three months after discontinuance of the CCl_4 injections the hepatic cells in the livers of these rats appeared histologically normal and very little fibrosis was present. At this time the mean vitamin A concentration in the livers was found to be 12.5 ± 8.6 international units per gram of fresh tissue. The livers of 6 control rats receiving the same amount of food and of vitamin A as the CCl_4 treated rats had a mean vitamin A concentration of 26.9 ± 9 I.U./g. The difference between these values was found to have statistical significance.

No correlation was observed between the vitamin A concentration in the cirrhotic livers and (1) the liver weight, or (2) the degree of fibrosis, or (3) the brewers' yeast intake of the animals.

It is concluded that the livers of rats recovering from CCl_4 cirrhosis contain much less vitamin A than do the livers of normal rats on the same diet.

---

[22] Wendt, H., and König, D., *Klin. Wochenschr.*, 1937, **16**, 1253.

[23] Cox, A. J., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 333.

[24] Lasch, F., and Roller, D., *Klin. Wochenschr.*, 1936, **15**, 1636.

13436

Purification of Paralytic Shellfish Poison by Filtration Through Active Charcoal.

WILLIAM M. BENDIEN AND HERMANN SOMMER. (Introduced by K. F. Meyer.)

From the George Williams Hooper Foundation, University of California, San Francisco.

In previous attempts to isolate the paralytic shellfish poison[1-5] various adsorbents and base exchange materials proved helpful for the purification of this highly potent organic base. Mueller[3] made use of Norit to remove pigments from acid alcoholic solutions of the poison; under these conditions the active substance is found quantitatively in the filtrate. Monnier[4] found that Norit adsorbs the poison completely from a neutral aqueous solution. Difficulties in eluting the substance quantitatively with acid indicated that this adsorption was not a simple base-exchange reaction. More detailed investigations now reveal that, besides the base exchange, a chromatographic type of adsorption is involved which can be utilized to effect a considerable purification of the poison, as the following examples show.

A poison solution was first used which had been partly purified by Monnier[4] by filtration through permutit and consequently contained mostly salts of strongly basic substances. The solution was adjusted to 150 lethal mouse units per cc and an acidity of 1 n HCl. When such a solution is filtered through a column of purified Norit A the filtrate is practically free of poison. Nor does the washing with a few cc of water remove appreciable amounts of the active substance. On further washing with water, however, practically all of the poison is eluted, in a greatly purified form. Traces of acid present obviously prevent a base exchange adsorption on the charcoal. A typical example is given in Table I.

Crude mussel extracts can also be purified by this procedure, provided they contain 150 MU per cc. The mussel livers are extracted with acid methyl alcohol (0.04 n HCl). Filtration through Norit now only removes the pigments and colloids. The resulting

---

[1] Meyer, K. F., Sommer, H., and Schoenholz, P., *J. Prev. Med.*, 1928, **2**, 365.

[2] Prinzmetal, M., Sommer, H., and Leake, C. D., *J. Pharmacol.*, 1932, **46**, 63.

[3] Mueller, H., *J. Pharmacol.*, 1935, **53**, 67.

[4] Monnier, R. P., Thesis, Eidgen. Techn. Hochsch., Zürich, 1938.

[5] Sommer and Monnier, unpublished data.

TABLE I.

Purification of Shellfish Poison by Filtration through Norit. Dimensions of column: 80x11 mm; rate of filtration approx. ½ cc per min.

	Vol. cc	Acidity, n	Concentration, MU* per cc	Amount, MU	Purity, γ per MU	Yield, %
Starting material	20	1.0	150	3000	25	100
Filtrate	20		5	100		3
Wash water	5	0.5	5	25		1
Eluate I (water)	10	0.1	265	2650	3.3	93
Eluate II "	5		30	150		

*MU—Lethal mouse units.

colorless solution is evaporated to dryness, the residue taken up in aqueous HCl, 1 n and purified as above. With mussels in which the poison content is high compared to the pigments and colloids the preliminary purification can be omitted. By a single filtration of the acid aqueous solution the pigments are removed by irreversible adsorption, the salts and other inert electrolytes are discarded in the filtrate and the poison is concentrated in the eluate. By repetition of the procedure under identical conditions an over-all yield of 70% and a 10-fold purification (from 50 γ to 4.7 γ per MU) are obtained. The saving of time and precious material by this method is evident as compared with other procedures.

The following analytical tests were made with poison samples of a potency of 3.5 γ per MU, which are comparable to the best products obtained by Monnier.[4]

Fluorescence. The products obtained showed no fluorescence in the ultraviolet, in contrast to some of the crude poison preparations. On detoxification with boiling alkali the fluorescent substance reappears, indicating that this is an atoxic transformation product of the poison.

Ash. Since the inorganic constituents are much less retarded by the filtration than the toxic substance the resulting products are low in ash. In 2 cases 2.4 ± 0.6% and 0.0 ± 0.5% ash were obtained.

Nitrogen and Chlorine. Determinations of nitrogen (by micro-Kjeldahl) and chlorine in different samples showed from 7.0 to 7.5% and 17.5 to 19.8% respectively, giving in all cases one chlorine ion for each atom of nitrogen. This indicates that all the nitrogen present is of basic nature.

Test with Nessler's Reagent. Of the various reagents tested only Nessler's solution was found to give a positive test. A greenish yellow coloration and turbidity are obtained, resembling strongly the test obtained with aliphatic diamines. Further purification experiments will have to show whether the test is specific for the poison or due to an accompanying impurity.

Summary. A simple and efficient method for the purification of shellfish poison consists in differential retardation when the poison is filtered through a charcoal column. The resulting products are low in ash, have lost their fluorescence, have an equal number of Cl and N atoms and give a characteristic Nessler reaction.

After completion of this work the article by Tiselius (*Science,* 1941, **94**, 145) came to our attention in which the author demonstrated the differential retardation of various salts, sugars, amino acids and peptides through active carbon and other adsorbents and proposes the procedure as a method of analysis of wide application. The separation of the colorless substances is, in this case, made visible by the Schlieren method.

13437 P

Production of Anemia by Tetanus Toxin.

HANNAH FARKAS AND I. J. KLIGLER.
From the Department of Hygiene and Bacteriology, Hebrew University, Jerusalem.

Until the recent extensive studies by Abel[1] it was generally accepted that tetanus toxin reaches the central nervous system by way of the nerve tracts. This view was based largely on the experimental work of Marie, Marie and Morax and others.[2] However, the experiments of Abel and his associates[1] showed that the toxin was distributed through the body by way of the lymphatic and blood systems, the specific effect of the toxin being produced on the nerve tissue with which it came in contact. Even prior to Abel's work, Van den Reis[3] according to Weinberg suggested that tetanus toxin caused a secondary anemia.

The object of the experiments reported below was to study the effect of tetanus toxin on the blood. The experiments were made on rabbits which in contrast with guinea pigs, rats and mice gave a constant red cell count. Individual normal rabbits examined regularly during a period of one month showed only slight variations in total red cell count, hemoglobin index and sensitivity to hypotonic salt solutions.

---

[1] Abel, J. J., *Science,* 1934, **79**, 63, 121; *Bull. Johns Hopk. Hosp.,* 1935a, **56**, 84, 317; 1935, **57**, 343.

[2] Marie, A., *Ann. Inst. Pasteur,* 1897, **11**, 591; 1898, **12**, 91; Marie, A., and Morax, V., *Ann. Inst. Pasteur,* 1902, **16**, 818; Meyer, H., and Ransom, F., *Arch. Path. Pharm.,* 1903, **49**, 369.

[3] Van den Reis, *K. W.,* 1922, **1**, 950.

The effect of the toxin was determined by the changes resulting in the red cell count, the hemoglobin index (Talquist), sedimentation rate and lysis, subsequent to the injection of toxin. The white cell counts showed no constant changes and were, therefore, not taken into account. Graded sublethal doses were injected intravenously or intramuscularly and the blood changes noted beginning with 1 hour after the injection and for varying periods thereafter. The potency of the toxin was titrated on mice according to Behring's method and the M.L.D. for rabbits based on VonLingesheim and Sherrington's[4] calculations that the specific resistance of rabbits was twice that of mice.

The results of a comparable series of experiments with varying doses of toxin given i.v. and i.m. respectively, are summarized in Table I. It will be seen from the data in the table that doses as low as 0.3 M.L.D. produced marked anemia; that the degree of change was approximately the same with the doses used, but that the rate of damage was dependent on the dosage; that the same dose given i.m. acted more slowly than when given i.v. In all instances the direction of change was the same—a marked decrease in the number of red cells, 50-60%; a corresponding decrease in hemoglobin; a significant increase in the sedimentation rate.

In order to ascertain whether the anemia was due to a specific effect of the toxin, rabbits showing a marked decrease in red cells following the injection of toxin, were given an injection of antitoxin. The effect was prompt and striking; after 2 hours the number of red cells rose by 50% and after 24 hours the count was nearly normal; the hemoglobin and sedimentation results followed a parallel course.

Reticulocyte counts at different phases verified the assumption that the toxin produced a type of secondary anemia which was resolved by the injection of antitoxin. The reticulocyte counts were made by staining with brilliant cresyl blue. In one series of counts the following results were obtained:

Before injection of toxin	4-5 *	reticulocytes
After injection of toxin	6-9	”
2 hours after injection of antitoxin	15-34	”
16 hours after injection of antitoxin	51	”

*Per million.

It seems that either the toxin destroys the reticulocytes as they

---

[4] Lingelsheim, von. See Kolle and Wasserman's *Hdb. Path. Mikroorg.*, 1912, 2te Abt., 1912-13, **4**, 737; Sherrington, C. S., *Lancet*, 1917, **2**, 964.

TABLE I.

No. of exp.	Mode of injection	Dose	No. of R.B.C.		Time,* h	Hemoglobin		Time,* h	Sedimentation		
			At start	Minimum		At start	Minimum		At start, mm	Minimum, mm	Time,* h
1	i.v.	0.58	5,340,000	2,180,000	21	80	40	4.5	2	4	21
1′	i.m.	0.58	5,780,000	3,420,000	144	61	45	144	1.5	4	144
2	i.v.	0.49	4,250,000	2,000,000	46	65	45	46			
3	i.v.	0.48	3,540,000	1,100,000	2	60	35	72	2	3	48
3′	i.m.	0.47	3,750,000	2,100,000	48	65	45	48	2	3	48
4	i.v.	0.46	3,750,000	1,850,000	25	65	45	5			
4′	i.m.	0.41	5,300,000	2,940,000	144	75	50	192	2		
5	i.v.	0.3	4,600,000	1,900,000	46	75	45	25			
6	i.v.	0.27	4,200,000	2,190,000	22	65	35	22	1.5	6	22
7	i.v.	0.1	4,520,000	3,780,000	51	75	70	51	3	3.5	27
7′	i.m.	0.1	4,250,000	3,780,000	48	80	75	6	3	3	

*Time elapsed after injection when minimum was reached.

enter the blood stream or else blocks the hematopoietic system. As soon as the antitoxin is administered the reticulocytes pour into the circulation and take the place of the destroyed red cells. This accounts for the dramatic restoration of the count within two hours after the injection of the antitoxin.

While this investigation was in progress Schwarzmann[5] showed that the toxin combines with the hemoglobin of the red corpuscles. Preliminary experiments were made to ascertain what portion of the toxin injected was present in the blood. Two rabbits were injected 13 M.L.D. of toxin, one i.v., the other i.m. Before death the rabbits were bled and the toxin content of the blood titrated by injecting varying dilutions into mice. From the results it was computed that the blood of the rabbit which received the toxin i.v. contained 40% and that of the rabbit injected i.m. 13% of the amount of toxin given.

Summary. Small sublethal doses of tetanus toxin produce a typical secondary anemia—low red cell count, reduced hemoglobin index, increased sedimentation rate. Injection of antitoxin produced a prompt return to normal, the red cell count rose rapidly as a result of large numbers of reticulocytes pouring into the system.

The severe anemia following the administration of toxin and the prompt recovery following that of antitoxin may serve as a simple early diagnostic test of possible tetanus intoxication in wounded.

---

[5] Schwarzmann, G., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 112.

13438

Cardiovascular Effects of Renin.*

OTTO SCHALES, SIBLEY W. HOOBLER AND FLORENCE W. HAYNES.
(Introduced by Soma Weiss.)

From the Medical Clinic, Peter Bent Brigham Hospital, and the Department of Medicine, Harvard Medical School, Boston.

Renin, a pressor protein from the kidney which has been suggested as a cause of clinical hypertension, raises the blood pressure largely through generalized peripheral vasoconstriction.[1, 2] During the pressor response, no significant changes have been observed in the skin temperature[3, 4] nor cardiac output[5] of animals. Hessel[6] reported the production of permanent hypertension in rabbits by repeated intravenous injections of pig renin.

The effects of renin on human beings have not been extensively studied but it has been shown that hog renin is ineffective[7, 8] while human renin raises the blood pressure.[7] Angiotonin, formed by the action of renin on blood globulins, has been shown to increase the arterial and venous pressure and to decrease the heart rate, cardiac output and blood flow in the limbs in human beings.[9, 10]

Methods. In the present investigation extracts of human and hog kidneys were prepared and assayed by methods described elsewhere.[11, 12] A rabbit unit has been defined as the amount of renin (expressed as micrograms nitrogen) per kilogram of body weight which would cause an average rise in arterial blood pressure of 30 mm mercury.[11] A human unit is similarly defined as the amount of renin per kilogram of body weight required to produce in human beings a rise of 30 mm mercury in systolic arterial pressure. In un-

---

* Aided in part by a grant from the John and Mary R. Markle Foundation.

[1] Tigerstedt, R., and Bergman, P. G., *Skand. Arch. Physiol.*, 1898, **8**, 223.

[2] Leiter, L., *Ann. Rev. Physiol.*, 1941, **3**, 509.

[3] Landis, E. M., Montgomery, H., and Sparkman, D., *J. Clin. Invest.*, 1938, **17**, 189.

[4] Corcoran, A. C., and Page, I. H., *Am. J. Physiol.*, 1939, **126**, 354.

[5] Somkin, E., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 200.

[6] Hessel, G., *Klin. Wchnschr.*, 1938, **17**, 843.

[7] Battro, A., Braun-Menendez, E., Lanari, A., and Leloir, L. F., *Rev. Soc. Argent. Biol.*, 1940, **16**, 376.

[8] Turnoff, D., and Rowntree, L. G., *Science*, 1941, **93**, 281.

[9] Wilkins, R. W., and Duncan, C. N., *J. Clin. Invest.*, 1941, **20**, 721.

[10] Bradley, S. E., and Parker, B. A., *J. Clin. Invest.*, 1941, **20**, 715.

[11] Schales, O., and Haynes, F. W., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 315.

[12] Schales, O., *J. Am. Chem. Soc.*, in press.

anesthetized dogs the blood pressure was measured by direct arterial puncture. In human beings the blood pressure was measured by the auscultatory method, blood flow in the hand or forearm by plethysmographic methods,[13] the venous pressure by the direct method of Moritz and von Tabora,[14] and cardiac output by the acetylene method.[15] All extracts were injected intravenously, the total dose being given during a period of one minute.

Results. Specificity of Renin. Hog renin was found to be equally active in rabbits and dogs, but inactive in human beings (no pressor response with 13 rabbit units per kg body weight). In order to produce the same rise in blood pressure in dogs as in human beings, 1-3 human units of human renin were required per kg dog. Rabbits required 5-15 times as much human renin per kg as human beings in order to show the same effect. The rise in blood pressure in rabbits was slower and lasted longer after human than after hog renin.

Effect of Repeated Injections of Hog Renin in Rabbits. After a control period of 3 weeks each of two rabbits was given one single daily dose of 2-3 R.U. per kg for 16 days. This treatment did not produce any permanent change in blood pressure. Two other rabbits received 4-6 injections (5-7 R.U. per kg each) daily for 3-4 weeks. During the injection period, the rabbits ate less and one finally refused all food and died at the 22nd day. The kidneys of this animal were grossly and microscopically normal except for a slight thinning of the cortex. During the second half of the injection period the sensitivity of both animals to one rabbit unit had decreased to about one-third of that of untreated rabbits. Doses of 2 R.U./kg and more were often followed by albuminuria. In the electrocardiograms of rabbits receiving repeated doses of renin a marked decrease in the heart rate and an inversion of T_2 or T_3 appeared shortly after large single doses. Similar records on rabbits not receiving daily injections also showed bradycardia as well as auricular premature beats after doses greater than about 5 R.U. Permanent hypertension as reported by Hessel[6] was not obtained.

Effect of Human Renin on Human Beings. In 14 male persons with normal blood pressure and 6 hypertensive patients, human renin consistently produced a rise in blood pressure which was approximately proportional to the dose of extract administered. In the best extracts, the amount of renin obtained from 0.05 g of human kidney, injected in one minute, was sufficient per kg body weight to raise the blood pressure 30 mm. The amount of renin recovered from kidneys of patients with severe hypertension was not significantly different from the amount obtained from normal kidneys; similar data have been reported before.[16, 17] The response began 1 to 2 minutes after the start of the injection, reached a maximum in 4-5 minutes and returned to the previous level in 20 to 30 minutes. Elevation occurred in both systolic and diastolic levels, but at higher dosages the systolic rise was usually greater. The largest dose given was 2.3 human units per kg and it produced a rise of 73 mm systolic and 31 mm diastolic pressure. Six mildly hypertensive patients, when compared with normal persons of similar age, exhibited no consistent increase in sensitivity to the extracts. Slight temporary fullness of the head, tightness of the chest and cold extremities after large doses were the only symptoms experienced. The test for albumin in 2 urine samples was negative. Twenty-seven injections of varying amounts of human renin in one normal person, did not significantly change his pressor response to this material.

---

[13] Kunkel, P., Stead, E. A., Jr., and Weiss, S., *J. Clin. Invest.*, 1939, **18**, 225.

[14] Moritz, F., and von Tabora, D., *Deutsches Arch. f. klin. Med.*, 1909-10, **98**, 475.

[15] Grollman, A., Friedman, B., Clark, G., and Harrison, T. R., *J. Clin. Invest.*, 1933, **12**, 751.

With moderate blood pressure elevation a drop in heart rate regularly occurred, in one instance falling from 66 to 40. Electrocardiograms, taken on 9 occasions, showed sinus bradycardia and, in 3 instances, there was a slight increase in the amplitude of the T-waves. The cardiac output in one normal person (3 experiments) showed a decrease of 0.2 liters/minute which was judged to be of no significance. Simultaneously the B.P. rose 42-56 mm systolic and 36-42 mm diastolic and the stroke volume increased 2-9 cc per minute as the pulse rate fell. In 3 experiments the basal metabolic rate did not change significantly. In 2 normal persons the blood flow in the hand at 40°C fell from an average of 27 cc to 3 cc per minute per 100 cc of tissue during a pressor response of 43-67 mm in systolic blood pressure. The volume of the hand decreased after the injection of renin. In 3 persons the forearm blood flow at 32°C did not change or decreased only slightly after renin, whereas adrenalin, 0.4 cc of a solution 1:20,000 intravenously, caused a sharp transitory increase in flow. After renin venous pressures of 3 normal persons rose 8-83 mm water concurrently with rises of 13-54 mm mercury in systolic pressure (5 experiments).

The observations reported here correspond in general with those of other investigators on the action of angiotonin in human beings,

---

[16] Prinzmetal, M., Friedman, B., and Abramson, D. I., *Ann. Int. Med.*, 1939, **12**, 1604.

[17] Landis, E. M., *Am. J. Med. Sci.*, 1941, **202**, 14.

except that angiotonin is reported to cause a reduction in cardiac output when measured by the ballistocardiographic method.[9, 10] The acute pressor effect of renin differs from that of adrenalin in its failure to increase the cardiac output, basal metabolic rate[18] and forearm flow. In these respects the action of renin is similar to that of paredrinol[19] but renin produces a more marked decrease in the blood flow of the hand than does paredrinol. The acute effect of renin differs from chronic hypertension in man both by the elevation of venous pressure and the decrease in blood flow in the hand.[20]

13439

Duality of Pituitary Gonadotrophins. Effects of FSH-ICSH Mixture on Hypophysectomized Male and Female Rats.*

H. FRAENKEL-CONRAT, C. H. LI, M. E. SIMPSON AND H. M. EVANS.
From the Institute of Experimental Biology, University of California, Berkeley, California.

The dual nature of the gonadotrophins of pig and sheep pituitaries has been demonstrated beyond question by the preparation of pure ICSH† [1, 2, 3] (interstitial cell stimulating or luteinizing hormone) and very active[4, 5, 6] or biologically pure[7, 8] FSH† (follicle-stimulat-

---

[18] Grollman, A., *The Cardiac Output of Man in Health and Disease*, Chas. C. Thomas, 1932.

[19] Stead, E. A., Jr., and Kunkel, P., *J. Clin. Invest.*, 1939, **18**, 439.

[20] Weiss, S., Publication No. 13, *Blood, Heart and Circulation*, Am. Assn. Adv. Sci., 1940, p. 295.

* Aided by grants from the Board of Research of the University of California and the Rockefeller Foundation, New York City, and Parke, Davis and Company, Detroit, Mich. We wish to acknowledge assistance from the Work Projects Administration, Project No. OP-65-1-08, Unit A-5.

† The use of names which clearly indicate a function of a hormone such as follicle stimulating (FSH) or gametotropic on the one side and interstitial cell stimulating (ICSH) or hormonotropic on the other appears to us preferable to the coinage of a new nomenclature.

[1] Shedlovsky, T., Rothen, A., Greep, R. O., Van Dyke, H. B., and Chow, B. F., *Science*, 1940, **92**, 178.

[2] Li, C. H., Simpson, M. E., and Evans, H. M., *Endocrinology*, 1940, **27**, 803.

[3] Li, C. H., Simpson, M. E., and Evans, H. M., *Science*, 1940, **92**, 355.

[4] Fraenkel-Conrat, H., Simpson, M. E., and Evans, H. M., *Anales de la Facultad de Medicina* (Montevideo), 1940, **24**, No. 5, 6, 7, 8.

[5] Fraenkel-Conrat, H., and Simpson, M. E., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 627.

[6] Jensen, H., Tolksdorf, S., and Bauman, F., *J. Biol. Chem.*, 1940, **135**, 791.

[7] Greep, R. O., Van Dyke, H. B., and Chow, B. F., *J. Biol. Chem.*, 1940, **133**, 289.

[8] McShan, W. H., and Meyer, R. K., *J. Biol. Chem.*, 1940, **135**, 473.

ing hormone) from both sources. The results of certain parabiotic experiments, however, have in the opinion of some authors cast doubt on the *secretion* of two gonadotrophins by the pituitary.[9]

The most clear-cut of these experiments was performed by Greep.[10] He united parabiotically with a castrate male both a hypophysectomized male and female rat and thus was able to observe the effects of the same pituitary acting simultaneously on the male and female gonads and accessories. He noted follicular and uterine development in the female, and testis and accessory enlargement in the male parabiont. This has been looked upon as evidence for the view that only one hormone is actually secreted by the pituitary which in this case showed the properties attributed to FSH in the female and to ICSH in the male rat.

Known differences in the reactivity of the two sexes to the two pituitary gonadotrophins appeared to us to amply account for the results of such parabiotic experiments. The effort was hence made to demonstrate that these diverse effects on a male and a female parabiont could be reproduced by administering the same mixture of the two purified gonadotrophins to hypophysectomized males and females.

A mixture of 0.5 units of FSH and 0.5 units of ICSH therefore was injected subcutaneously daily for 4 days to 2-day post-operative 23-day-old hypophysectomized male and female rats, with autopsies on the 5th day, 24 hours after the last injection. As indicated in Table I, testis and ventral prostate enlargement‡ were evident in the male rats, while follicular development and estrous uteri but no corpora lutea occurred in the females.§ It has thus been shown that

---

[9] Smith, P. E., *Univ. of Pennsylvania Bicentennial Conference,* 1941, 19-31.

[10] Greep, R. O., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 214.

‡ Confirming the interesting discovery of Greep *et al.*,[11] we find the ventral prostate of the 2-day postoperative, 23-day-old rat very much more sensitive as an indication of the action of ICSH than any other part of the male accessories, such as the seminal vesicles.[12] This type of rat has therefore been routinely used for the assay of ICSH and for that reason also in these experiments.

[11] Greep, R. O., Van Dyke, H. B., and Chow, B. F., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 644.

[12] Fraenkel-Conrat, H., Li, C. H., Simpson, M. E., and Evans, H. M., *Endocrinology,* 1940, **27**, 793.

§ Histological examination of the ovaries of rats receiving the two gonadotrophins also revealed only follicles with no signs of luteinization. The interstitial tissue, which in controls of both sexes does not show fargoing regression during this short postoperative period, did not appear appreciably stimulated by the ICSH alone or in combination with the FSH. This is in accord with previous findings that somewhat smaller amounts of ICSH can be detected, on subcutaneous injection, by its augmenting effect on the FSH, in the female, than are needed for repair or

TABLE I.
Weights of Gonads and Accessories of Hypophysectomized Male and Female Rats* Injected with FSH and ICSH†

Hormones	No. of rats		Males			Females	
	♂	♀	Testes, mg	Ventral prostate, mg	Seminal vesicles, mg	Ovaries, mg	Uterus, mg
FSH + ICSH	9	10	224 (195-280)	13 (10-15)	7 (4-10)	24‡ (13-31)	80‡ (53-103)
FSH alone	5	3	242 (236-249)	8 (6-8)	7 (6-8)	12 (10-14)	22 (18-28)
ICSH alone	5	4	130 (118-154)	12 (10-15)	8 (5-11)	10 (6-12)	21 (20-22)
Controls	4	3	117 (103-124)	8 (7-8)	7 (6-9)	9 (8-10)	24 (20-31)

*23 days old, 2 days p.o. at onset of injections, injected subcutaneously daily for 4 days, with autopsies on 5th day.

†A total dose of 2 units was given of each hormone, with the exception of the group of males receiving FSH alone, in which the dose was doubled to test for ICSH contamination. Units were determined in the usual 3-day test in 6-8 day post-operative rats, 26-28 days old at operation.[1,4] Both preparations used contained 80 units per mg. The ICSH contained less than 0.1% FSH; the FSH about 10% ICSH.

‡Medium to medium large follicles, no luteinization; estrous uterus in 7 rats. No follicular or uterine stimulation in the other groups.

an artificial mixture of the two sheep gonadotrophins will be follicle-stimulating in its action on the female, while in the male it stimulates the interstitial tissue to secrete androgens. The logical interpretation of these findings appears to be that there is a difference in the responsiveness of the two sexes to the two hormones: the female being more sensitive to the FSH, the male to the ICSH. Nevertheless the presence of the ICSH is evident also in the female (in our as well as in Greep's experiment), by its augmentation of the follicular-stimulating effect of the FSH and by the production of estrogens, now believed to be due to the synergism of the two hormones.[12, 13, 3] Conversely, the presence of the FSH also documents itself in the male by the considerable enlargement of the testes which is far greater than that produced by the ICSH alone.[‖]

This response of the two sexes to the same gonadotrophic mix-

---

stimulation of the interstitial tissue. Similarly the FSH alone showed no activity at this level and in this type of rat, while assays in the usual type of hypophysectomized rat showed the indicated potency.

[13] Fevold, H. L., *Endocrinology*, 1940, **26**, 999.

[‖] The effect is additive; no augmentation of testis or accessory weights was observed in this nor in many other experiments in which both FSH and ICSH were given to hypophysectomized male rats.

ture resembles qualitatively, though obviously not quantitatively, that noted by Greep in his male and female parabionts, both under the stimulation of one pituitary. Parabiotic studies can thus not be regarded as evidence concerning the number of gonadotrophins secreted by the pituitary. Such evidence can be obtained only from the isolation of the pure hormones from the blood as it has been done from the pituitary.

Summary. A mixture of purified FSH and ICSH was shown to produce predominantly follicle-stimulating effects in female and interstitial cell-stimulating effects in male hypophysectomized rats. These differences in the response of the two sexes were secured by the simultaneous administration of both hormones and it would consequently not seem valid to interpret such phenomena as evidence for the existence of a single pituitary gonadotrophin.

13440 P

Nitrogen Balance in Experimental Tryptophane Deficiency in Man.*

L. EMMETT HOLT, JR., ANTHONY A. ALBANESE, JOSEPH E. BRUMBACK, JR., CHARLOTTE KAJDI AND DOROTHY M. WANGERIN.

From the Harriet Lane Home, Johns Hopkins Hospital, and the Department of Pediatrics, Johns Hopkins University, Baltimore, Md.

The observations here reported comprise a part of a more comprehensive study undertaken jointly by the Departments of Medicine and Pediatrics of the Johns Hopkins University on experimental amino acid deficiencies in man. The purpose of this study was twofold. It was felt desirable to determine human amino acid requirements by direct observations on man, rather than by inferences based on animal experiments, since it is now known that even closely related animal species differ in their amino acid requirements. A second purpose was to discover whether deficiencies of particular amino acids produced characteristic pathological changes which could be recognized by clinical or laboratory technics. It seemed probable that, in certain parts of the world at least, specific amino acid deficiencies might be present, and that a familiarity with the picture, such as could be obtained in an experimental study, would be of aid in recognizing and treating them.

---

* The expenses of this study were defrayed by grants from the Rockefeller Foundation, Merck and Company, Mead Johnson and Company, Eli Lilly and Company, and E. R. Squibb and Sons.

The present report deals with the nitrogen balance of 4 normal adult males, who ingested a diet deficient in tryptophane for periods of 2 to 5 weeks. The experimental diet consisted of fats, starch, sugar and certain fruits and vegetables selected for their low protein content (tomatoes, mushrooms, carrots, asparagus, apples, oranges, etc.). Of the total nitrogen ingested only 10% was in the form of food proteins, the remaining 90% being supplied as a mixture of amino acids devoid of tryptophane (an acid hydrolysate of casein to which 1% cystine was added). The total nitrogen intake was maintained throughout the experiment at 0.1 g per kilo per day. The intake of non-nitrogenous food was identical for all subjects, providing approximately 800 calories of fat and 1500 calories of carbohydrate per day. The total caloric intake varied in the different subjects between 2400 and 2700 calories per day. A daily vitamin supplement of one-fourth of a Merck's No. 1 TRN6P tablet was given to each individual.

For a week preceding the experimental period and for a week following it the subjects were placed on a diet identical with that of the experimental period except that it contained tryptophane. The foreperiod served to adapt the subject to a diet somewhat different from his customary one which as a rule had contained more nitrogenous food. The post-period served as a control. The amino acid mixture of the control diet in subjects Br. and Bi. was identical with that used in the experimental period except that it was supplemented with 1.5% of tryptophane. The total nitrogen intake remained the same. In the case of the other 2 subjects the amino acid mixture in the control period consisted of an enzymatic casein hydrolysate in which the original tryptophane had not been destroyed. This, too, was enriched with 1% of cystine.

Throughout the experimental period the subjects retained a good appetite. They lived essentially normal lives and slept well. They were studied at intervals by a variety of clinical and laboratory technics with results which are being reported elsewhere.[1] The food and excreta were analyzed for nitrogen throughout the experiment, and balances were calculated for each two-day period. The course of the nitrogen balance for each subject is shown in Fig. 1.

It is apparent that each of the subjects went into negative nitrogen balance a few days after the tryptophane deficient diet was insti-

---

[1] Holt, L. E., Jr., Wintrobe, M. M., Albanese, A. A., Guild, S. R., Loch, Walter, Buschke, W., Fleischmann, W., and Lyddane, Anne, to be published.

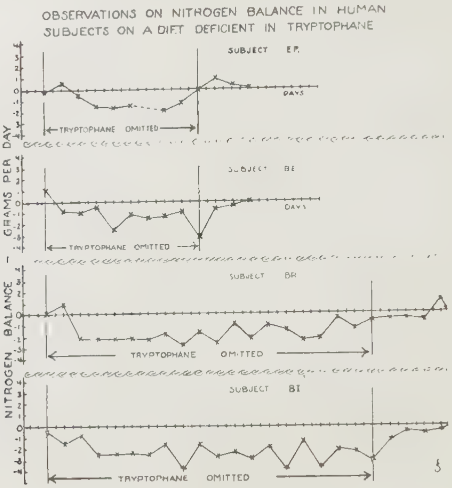


FIG. 1.

tuted, and that within a week after the restoration of tryptophane to the diet, nitrogen equilibrium was restored.

It appears, therefore, that nitrogen equilibrium cannot be maintained unless tryptophane is supplied in the diet, and that tryptophane must therefore be regarded as a dietary essential for the human species.[†]

13441 P

Nitrogen Balance in Experimental Lysine Deficiency in Man.*

ANTHONY A. ALBANESE, L. EMMETT HOLT, JR., JOSEPH E. BRUMBACK, JR., MARJORIE HAYES, CHARLOTTE KAJDI AND DOROTHY M. WANGERIN.

From the Harriet Lane Home, Johns Hopkins Hospital, and the Department of Pediatrics, Johns Hopkins University, Baltimore, Md.

As part of a general study designed to determine the amino acids essential for man, the nitrogen balance was studied in 5 human volunteers who were given a diet deficient in lysine for periods of 2 to 5 weeks in length. The basic diet and the conditions of the experiment were similar to those outlined in the accompanying report[1] on tryptophane deficiency. The amino acid mixture deficient in lysine was prepared by deamination of casein, a process which has been shown to destroy the biological activity of lysine;[2] this was followed by acid hydrolysis, with the addition of 1.5% tryptophane (to replace that lost during hydrolysis) and of 1% cystine.

[†] We have been unable to find in the literature observations dealing with tryptophane deficiency in man, but we have recently had the privilege of examining the data from a single unpublished experiment performed by Dr. Warren M. Cox, Jr., in which a tryptophane deficient diet, continued for 4 days, induced a negative nitrogen balance. (Cox, W. M., Jr., Mueller, A. J., and Fickas, D., to be published.)

* The expenses of this study were defrayed by grants from the Rockefeller Foundation, Merck and Company, Eli Lilly and Company, and E. R. Squibb and Sons.

Before the deficient diet was instituted the subjects were given a diet identical with the experimental diet except that an amino acid mixture containing lysine was supplied. This mixture consisted of an enzymatic hydrolysate of casein, enriched by 1% cystine. A control period on an amino acid mixture containing lysine followed the deficiency period. In subjects Es. and Ha. the amino acid mixture in the control period was identical with that of the experimental period except that 6% lysine was added. In subjects Ra., He. and Hs., the amino acid mixture in the control period was identical with that used in the foreperiod.

The observations on nitrogen balance in 4 male subjects are shown in Fig. 1. It may be seen that each subject developed a negative nitrogen balance within a few days after the lysine-deficient diet was started, and that nitrogen equilibrium was promptly restored when lysine was again supplied. The failure to maintain nitrogen equili-

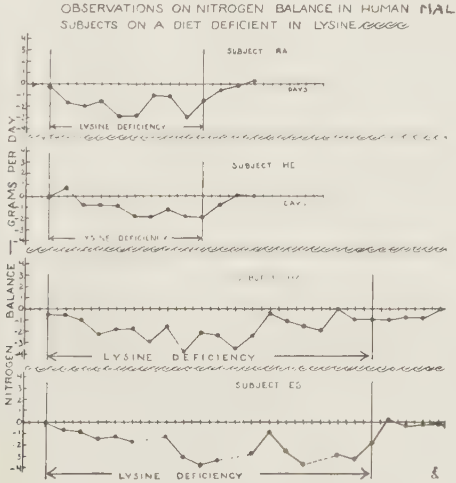


Fig. 1.

---

[1] Holt, L. E., Jr., Albanese, A. A., Brumback, J. B., Jr., Kajdi, C., and Wangerin, D. M., Proc. Soc. Exp. Biol. and Med., 1941, **48**, 726.

[2] Dunn, M. S., and Lewis, H. B., *J. Biol. Chem.*, 1921, **49**, 327.

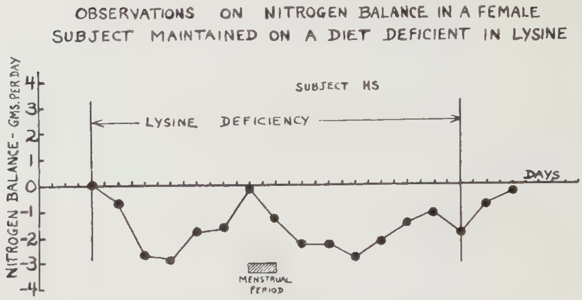


Fig. 2.

brium in the absence of an adequate supply of this amino acid indicates clearly that lysine is a dietary essential for the human subject.

The single experiment performed on a female subject (Fig. 2) also shows the prompt development of a negative nitrogen balance on withdrawal of lysine and the return to nitrogen equilibrium when lysine was restored. It may be noted, however, that on one occasion during the deficiency period the negative nitrogen balance virtually disappeared. This point coincided with the onset of menstruation. We are inclined to attribute this brief period of nitrogen equilibrium to a decrease in the metabolic requirements associated with menstruation, a phenomenon described by Benedict and others.[3] The menstrual period in our subject was characterized by an unusually scanty flow and by the complete absence of symptoms of premenstrual distress—swelling of the breasts, headache and gain in weight—which the subject usually experienced. This finding is of interest in view of the known influence of a lysine-deficient diet in suppressing the estrous cycle in animals.[4]

13442 P

Clotting Action of Rabbit-Clotting Globulin.

R. M. Bird, B. McSwain, G. L. Kauer, Jr., and Frank Glenn.
(Introduced by P. Reznikoff.)

From the Departments of Medicine and Surgery, New York Hospital, and Cornell University Medical College.

Parfentjev[1] has shown that a "pseudoglobulin" fraction derived from rabbit plasma acts as a powerful blood coagulant. Taylor and his coworkers[2, 3] have reported on its *in vitro* action and its local hemostatic effect on cutaneous and oral bleeding surfaces. This report covers a study of the *in vitro* and *in vivo* action of "rabbit-clotting globulin."*

---

[3] Pearson, P. B., *Am. J. Physiol.*, 1937, **118**, 786.

[4] Benedict, F. B., and Finn, M. D., *Am. J. Physiol.*, 1928, **86**, 59.

[1] Parfentjev, A., *Am. J. Med. Sc.*, 1941, **202**, 578.

The clotting properties of the stock globulin solution were tested on oxalated and heparinized normal dog blood, on fibrinogen solution, on oxalated 1% prothrombin dog blood, and on blood containing adrenalin. In all instances the globulin exhibited powerful thrombic activity. Oxalated blood was clotted more rapidly than heparinized blood. Neither low prothrombin nor adrenalin inhibited the speed of coagulation. Quantitatively our globulin was not as potent a coagulant as the material reported on by the previous observers.[2]

Twenty-two dogs were used to study the effects of globulin administered intravenously. The injection of 0.03 cc or more per kg resulted in the appearance of toxic signs, and 0.3 cc or more per kg was invariably lethal. Doses smaller than 0.3 cc/kg occasionally resulted in death and the severity of toxic signs varied with individual animals. Two of 3 animals were protected from lethal intravenous injection of globulin by the subcutaneous administration of adrenalin. A significant reduction in the clotting time followed the intravenous injection of 0.01 cc to 0.05 cc of globulin per kg. When larger quantities were injected slowly there resulted initially a fall in the clotting time followed by a prolongation. No constant variation in the dog's prothrombin or fibrinogen blood levels accompanied a fall in the clotting time, but one or both of these decreased with a negative phase of coagulation. Thus the intravenous administration of globulin resulted in a positive or negative phase, or both, depending on the amount and rapidity of the injection.

Globulin introduced by stomach tube caused a fall in the clotting time. The graph illustrates the effect on clotting time, prothrombin and fibrinogen of 1 cc of globulin per kg given orally to a dog. Seven dogs were given globulin orally in doses from 1 cc to 4 cc per kg. In no animal did the fasting stomach contents contain free HCl. A fall in the clotting time was observed in every instance. Hypoprothrombinemia to 60% resulted in 3 dogs which received 2 cc or more per kg. Fibrinogen was never altered significantly. In no animal were toxic signs observed. *In vitro*, gastric juice containing 10° free HCl

---

[2] Taylor, F. H. L., Lozner, Eugene L., Adams, Margaret A., *Am. J. Med. Sc.*, 1941, **202**, 585.

[3] Lozner, Eugene L., MacDonald, Harriet, Finland, Maxwell, and Taylor, F. H., *Am. J. Med. Sc.*, 1941, **202**, 593.

* "Rabbit-clotting globulin" was supplied by Lederle Laboratories.

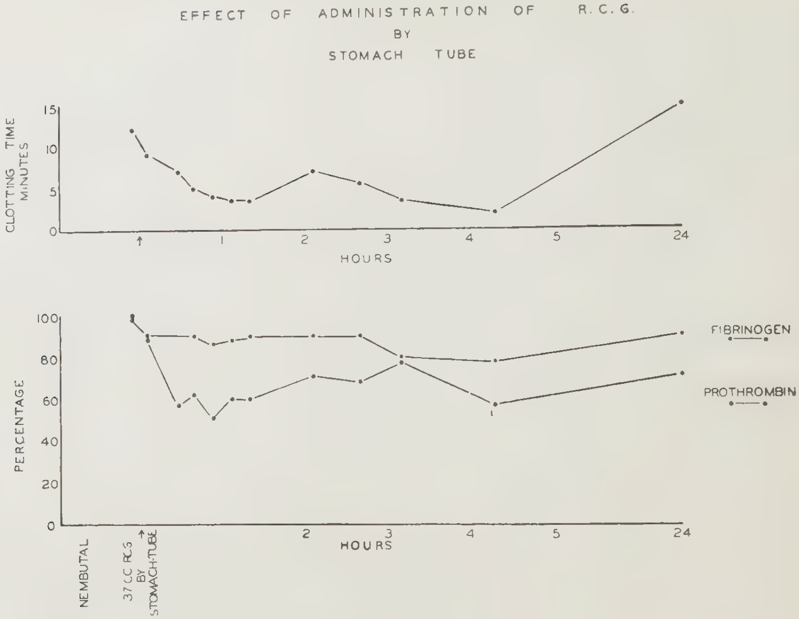


FIG. 1.

or N/10 HCl itself completely abolished the clotting action of the globulin even in high dilutions of the acid. Rectal instillation and subcutaneous or intramuscular injections were less effective in reducing the clotting time.

Local application to various cutaneous and visceral bleeding surfaces resulted in prompt hemostasis only in areas of capillary bleeding. Experimental pulmonary emboli were easily produced by release of the clot formed by injection of globulin into a temporarily ligated section of the inferior vena cava.

Eight patients with gastrointestinal hemorrhage or hemophilia have been given globulin by mouth with either cessation of bleeding or reduction in clotting time, or both, in 4 cases. This study is being continued.

AUTHORS' INDEX

(The numerals indicate the page.)

Abels, J. C., Gorham, A. T., Pack, G. T., and Rhoads, C. P. Vit. A, liver, cancer. 488.
Abramson, D. I., Landt, H., and Benjamin, J. E. Anoxia, vascular response. 214.
Abramson, M. 509.
Aebersold, P. C. 251.
Albanese, A. A., Holt, L. E., Jr., Brumback, J. E., Jr., Hayes, M., Kajdi, D., and Wangerin, D. M. Lysine deficiency, N balance. 726, 728.
Albanese, A. A. 726.
Allison, S. D. 476.
Almquist, H. J., and Mecchi, E. Tryptophane, chick needs. 526.
Alper, J. M. 478
Altschule, M. D., Linenthal H., and Zamcheck, N. Raynaud's disease, lung vol., cold. 503.
Alture-Werber, E. *Trypanosoma equiperdum* cultivation. 90.
Anderson, R. C. 676.
Ane, J. N., and Burch, G. E. Lymph flow, X-ray. 471.
Ansbacher, S., and Landy, M. Biotin, scaly dermatosis. 3, 118.
Apperly, F. L., and Cary, M. K. Hypertension, pulse pressure, arteriovenous O_2. 492.
Armstrong, W. D. 98.
Atkinson, W. B. 112.
Axelrod, D., Aebersold, P. C., and Lawrence, J. H. Tumors, X-rays, neutrons. 251.

Back, A. 150.
Baldwin, F. M., Wiswell, O. B., and Jankiewicz, H. A. Vit. K, hemorrhage control in *Eimeria tenella*. 278.
Ball, Z. B., and Barnes, R. H. Growth, lactation, dietary supplements. 692.
Barach, A. L. 346.
Barker, S. B. 237.
Barnes, R. H. 692.
Barrera, S. E. 596.
Barron, E. S. Guzman, and Goldinger, J. M. Sperm respiration, iodoacetate, malonate. 570.
Bartels, W. E. 540.
Beard, M. 566.
Beck, M. D. 177.
Bell, T. A., Satterfield, G. H., and Cook, F. W. Vit. C deficiency, leg weakness. 473.
Bender, M. B. 267, 447.
Benjamin, J. A. 263.
Benjamin, J. E. 214.
Bernhard, A., and Rosen, M. J. Blood, diastase distribution. 575.
Bigg, E. Pneumonia, specific sol. subst. 245.
Bing, R. J., Zucker, M. B, and Perkins, W. Renal extracts, angiotonin, tyramine. 372.
Birch, C. L. Estrogen, assay, hemophilia. 167.
Bird, R. M., McSwain, B., Kauer, G. L., and Glenn, F. Blood clotting globulin. 730.
Birnbaum, G. L. 203.
Bloomfield, A. L., and Lew, W. Cecitis, ulcerative, sulfaguanidine. 363.
Bogart, R. 217.
Bornstein, S. 359.
Boucher, R. V. 456.
Brechner, J., and Armstrong, W. D. Gastric acidity, bone resorption. 98.
Brightman, I. J. 116.
Brookman, B. T. 670.
Broun, G. O., Legier, M., Mezera, R. A., and Muether, R. O. Virus, encephalitis, immunology. 310, 357.
Brown, H. 138.
Brown, P. F. 284.
Brumback, J. E., Jr. 726, 728.
Bruner, D. W. 240.
Bruzzone, S. 467.
Burch, G. E. 471.
Burgin, C. J. 415.
Burstein, C. L., and Rovenstine, E. A. Paraldehyde, toxicity. 669.
Butcher, E. O. Adrenalectomy, hair growth, thyroid. 120.
Butler, E. G. 500.

Cannon, J. A. 628.
Carmichael, E. B. 381.
Carpenter, C. M., Charles, R., and Allison, S. D. Gonococcus, sulfathiazole. 476.
Cary, M. K. 492.
Catchpole, H. R. 133.
Chalecki, W. E., Witenberg, E. G., Taylor, J. D., and Hodge, H. C. Liver lipids, dinitrophenol. 302.
Chambers, L. A., and Henle, W. Virus, influenza A, protamine pption. 481.
Chang, K. P. S. 243.
Charipper, H. A. 169.
Charles, R. 476.
Chen, K. K., Anderson, R. C., and Robbins, E. B. Glucosides, cardiac. 676.
Chen, Y. P. Relapsing fever, expl. immunity. 48.
Chickering, O. 65.
Clisby, K. H. 684.
Cohen, S. S. Virus, tobacco mosaic, necrosis, sepn. 163.
Cole, K. S. 293.
Cole, W. H., and Nastuk, C. L. P*, disappearance, heart blood. 151.
Cone, N. M., Forman, S. E., and Krantz, J. C., Jr. Anesthetics, physical properties. 461.
Conrat, H. L. F., Herring, V. V., Simpson, M. E., and Evans, H. M.. Pancreas insulin, estrogen. 333.
Cook, F. W. 473.
Coombes, A. I. 376.
Cournand, A., Yarmush, I. G., and Riley, R. L. Respiration, O_2 breathing, N elimination, body size. 280.
Croxatto, H., and Croxatto, R. Hypertensin, amine-oxidase, tyrosinase action. 392.
Croxatto, R. 392.
Cunningham, B., and Kuhn, H. H. Androgens in placenta. 314.
Cunningham, R. W. 410.
Cutuly, E. Implantation, hypophysectomy, lactogenic hormone. 315.

Daft, F. S., Sebrell, W. H., and Lillie, R. D. Liver cirrhosis, dietary, expl. prevention. 228.
Dancey, T. E. 619.
Dann, W. J., and Handler, P. Nicotinuric acid, canine blacktongue. 355.
Davenport, H. W., and Wilhelmi, A. E. Carbonic anhydrase, renal. 53.

Davis, D. E., and Domm, L. V. Sexual behavior, intersexual fowl. 665; hormones, 667.
Diefendorf, H. W. Serum antibodies, str. hemolytic. 56.
Doan, C. A., Saslaw, S., Beard M., Woolpert, O. C., Schwab, J. L., and Merino, C. *Streptococcus hemolyticus*, reinoculation. 566; 558, 560, 563.
Domm, L. V. 665, 667.
Dosne, C. 352.
Doster-Virtue, M. E., and Virtue, R. W. Intestinal loop closure. 326.
Dragstedt, C. A. 152.
Dreisbach, R., and Snyder, F. F. Scopolamine, fetus. 197.
Drill, V. A. Hyperthyroidism, bone Ca. 448.
Dulaney, A. D., and House, V. Malaria, pption. tests. 620.
Dutcher, R. A. 456.

Eaton, M. D., and Beck, M. D. Virus, influenza B. 177, 181.
Eckman, M., and Barach, A. L. Gas sampling inspired air. 346.
Edwards, P. R., and Bruner, D. W. *Salmonella illinois*. 240.
Egana, E. 467.
Eichelberger, L., and Lindsay, J. R. Antrum cyst fluid, compn. 191.
Elvehjem, C. A. 349, 376.
Enders, J. F., and Pearson, H. E. Virus, influenza A, resistance. 143, 140.
Ercoli, N. Chemotherapeutic agent, osteotrophy. 672.
Evans, H. M. 333, 549, 634, 723.
Evans, T. C., Levin, A. J., and Sulkin, N. M. Embryo formation inhibition, X-rays. 624.

Farkas, H., and Kligler, I. J. Tetanus toxin, anemia. 717.
Fazekas, J. F. 446.
Fellows, E. J., and Cunningham, R. W. Cystine, bis-p-aminobenzoyl. 410.
Fellows, E. J. Sulfonamides, pyrimidine derivatives. 680.
Field, J., II. 423.
Figge, F. H. J., and Atkinson, W. B. Pantothenic acid deficiency, H_2O metabolism, porphyrin. 112.
Forbes, J. C. 361.
Forman, S. E. 461.
Fraenkel-Conrat, H., Li, C. H., Simpson, M. E., and Evans, H. M. Gonadotrophins, duality. 723.
Francis, T., Jr., and Brightman, I. J. Influenza, virus inactivation of nasal secretion. 116.
Frazier, C. N. 44.
Freeman, G. Virus, latent, immunity. 568.
French, A. J. Myocarditis, sulfonamides. 1.
Frey, C. N. 256.
Friedman, M. 429.
Fuhrman, F. A., and Field, J., II. Metabolism, tissue, triturus toxin. 423.
Fulton, G. P. Micromanipulator electrode. 427.

Gales, J. W. 95.
Gardner, W. U., and White, A. Mamma growth, hypophysectomy, estrogen, prolactin. 590.
Geiger, A. J., Monroe W. M., and Goodyer, A. V. N. Electrocardiography, fetal. 646.
Geist, S. H. 11.
Glenn, F. 730.
Goldhaber, G., and Back, A. Radiosensitivity—muscular and ciliary movement. 150.
Goldinger, J. M. 570.
Goodyer, A. V. N. 646.
Gordon, A. S. 169.
Gorham, A. T. 488.
Gottfried, S. P. 518.
Graves, R. W. 555.
Gray, S. W. Heart, ventricular filling. 131.
Grishman, A., and Master, A. M. Coronary occlusion, cardiac output. 207.
Guest, G. M. 451.
Gutman, A. B., and Gutman, E. B. Phosphorylase, calcifying cartilage. 687.
Gutman, E. B. 687.
Guttman, R., and Cole, K. S. Nerve, electrical rectification. 293.

Haag, H. B. 223.
Hammon, W. M., and Izumi, E. M. Virus, poliomyelitis, pH, "enteric organism filtrate." 579.
Handler, P. 355.
Handley, C. A., Sweeney, H. M., and Brookman, B. T. Metabolism, head, Na pentobarbital, metrazol. 670.
Hare, K. 158.
Hartman, C. G. Pregnancy, ovariectomy. 221.
Hartman, F. A., Lewis, L. A., and Thatcher, J. S. Sodium retaining subst., assay. 60.
Hayes, M. 728.
Haynes, F. W. 720.
Heilbrunn, G. 233.
Henderson, H. J., and Long, E. R. Tuberculosis, chlorin-e-rhodin-g. 435.
Henderson, H. J., and Long, E. R. Chlorophyll, visceral accumulation and elimination. 438.
Henle, W., and Zellat, J. Virus, influenza A propylene glycol aerosol. 544; 298, 481.
Henshaw, P. S. 74.
Heppel, L. A. Mannitol hexanitrate, pharmacology. 72.
Herring, V. V. 333.
Herrlich, H. C., Fazekas, J. F., and Himwich, H. E., Altitudes, infant, adult survival. 446.
Hess, W. C., Sullivan, M. X., and Palmes, E. D. Virus, tobacco mosaic, cystine. 353.
Heymann, W., and Wartman, W. B. Liver cells, vit. D. 274.
Himwich, H. E. 133, 446.
Hinsey, J. C., Hare, K., and Phillips, R. A. Nerve roots, spinal, skin temperature. 158.
Hinton, J. 330.
Hoagland, H. Blood vessels, acetylcholine sensitization. 326.
Hodge, H. C. 302.
Hogan, A. G., Muhrer, M. E., and Bogart, R. Hemophilia-like disease. 217, 459.
Holt, L. E., Jr., Albanese, A. A., Brumback, J. E., Jr., Kajdi, C., and Wangerin, D. M. Tryptophane deficiency, N balance. 726.
Holt, L. E., Jr. 413, 728.
Hoobler, S. W. 720.
Hood, R. T. 500.
Hooker, S. P. 69.
House, R. M., and Wakerlin, G. E. Blood pressure regulation, kidney. 513.
House, V. 620.
Howitt, B. F., and VanHerick, W. Virus, encephalitis, equine, antibodies. 247.
Hu, C. H. 226.
Hu, C. K., and Frazier, C. N. Testis atrophy, X-rays. 44.
Huang, C. H., and Sia, R. H. P. Sulfonamides, streptococci. 109.

Ivy, A. C. 9.
Izumi, E. M. 579.

Jacobs, J. L., Keiley, J. J., and Sommers, S. C. Sensitization, heredity. 639.
Jahn, T. L., and Wulff, V. J. Retinal pigment distribution, diurnal rhythm. 656. flicker response, 660.
Jankiewicz, H. A. 278.
Jewell, M. L. 27.
Johnson, F. H., and Moore, K. Luminescence, sulfonamide inhibition. 323.
Johnson, G. 516.
Johnson, T. U. 95.
Jones, H. P., Rake, G., and McKee, C. M. Lymphogranuloma venereum, sulfonamides. 318.

Kajdi, C. 726, 728.
Kaplan, D. 103.
Katzin, E. M. 126.
Kauer, G. L. 730.
Keiley, J. J. 639.
Kempf, A. H. 631.
Kempf, J. E., Pierce, M. E., and Soule, M. H. Virus, poliomyelitis, insulin and neurone injury. 187, 604.
Kensler, C. J., Young, N. F., and Rhoads, C. P. Glucuronic acid, liver, butter yellow. 22.
Kersten, H. 521.
Klatt, T. J. 403.
Kleiber, M. Metabolism, liver, body size. 419.
Kligler, I. J., and Kaplan, D. *Sp. gallinarum*, cultivation. 103, 717.
Knandel, H. C. 456.
Knotts, F. L. 129.
Kolmer, J. A., and Brown, H. Sulfonamides, expl. vaccinia. 138.
Kolmer, J. A. Sulfonamide, *B. diphtheriæ*. 390; and Rule, A. M. Sulfonamide, *B. typhosus*. 388.
Kramer, S. D. Virus, poliomyelitis, convalescent serum protection. 287.
Krantz, J. C., Jr. 461.
Krichesky, B. 263.
Krueger, E. 478.
Kueter, K. 403.
Kuhn, H. H. 314.
Kupferberg, A. B., and Johnson, G. *Trichomonas vaginalis*, estrogens. 516.
Kupperman, H. S., Mellish, C. H., and McShan, W. H. Serum, antigonadotropic. 79.

La Due, J. S. Digitalis, muscular weakness. 5.
Landt, H. 214.
Landy, M. 3.
Lalich, J. J. 616.
Law, L. W. Cancer, mamma, pseudopregnancy. 486.
Lawrence, J. H. 155, 251.
Leach, B. E., and Forbes, J. C. Carbon tetrachloride poisoning protection by sulfonamides. 361.
LeGier, M. 310, 357.
Levin, A. J. 624.
Levine, P., and Katzin, E. M. Erythroblastosis fetalis, pathogenesis. 126.
Lew, W. 363.
Lewis, A. A., and Turner, C. W. Hormone, lactogenic of pituitary, mamma, stilbestrol. 439.
Lewis, L. A. 60.
Lewis, S. R. 425.
Li, C. H. 723.
Liberson, W. T., and Strauss, H. Electroencephalogram, hyperventilation, age. 674.
Lichty, J. A., Jr., and Hooker, S. P. Erythrocyte sedimentation rate, acetyl salicylic acid, rheumatic fever. 69
Light, R. F., and Frey, C. N. Bone fractures, low Ca diet. 256.
Lillie, R. D. 228.
Lim, K. T. 199.
Lindsay, J. R. 191.
Linenthal, H. 503.
Lipschutz, A., and Vargas, L. Jr. Estrogens, tumors, age. 584; estrogen absorption, pellets, 464; Vargas, L. Jr., Egana, E., and Bruzzone, S. Estrogens, artificial, tumor. 467; Vargas, L., and Nunez, C. Antitumoral action steroid hormones. 271.
Liu, Y., and Hu, C. H. Tumors, expl., food tars. 226.
Loew, E. R., and Chickering, O. Gastric secretion, histamine antagonist. 65.
Long, E. R. 435, 438.
Lowenstein, O., and Bender, M. B. Nerve, optic, section, recovery pupillary function. 447.
Lyons, W. R., and McGinty, D. A. Mamma, progesterone. 83, 86; Simpson, M. E., and Evans, H. M. Hormone, lactogenic, mamma, vaginal opening. 634.

McCollum, E. V. 642.
McEwen, H. D., and MacLachlan, P. L. Phospholipid soly., digitonin. 195.
McGinty, D. A. 83.
McKee, C. M. 318.
McKenzie, F. F. 648, 654.
McShan, W. H. 79.
McSwain, B. 730.
Mackenzie, C. G., and McCollum, E. V. Vit. E. creatinuria, muscular dystrophy. 642.
MacLachlan, P. L. Blood lipids, fasting. 411, 195.
Main, R. J. Smoking, respiration, circulation. 495.
Major, R. H. 616.
Marburg, O., and Shwartzman, G. Brain, bacterial filtrate reactions. 612.
Marcuse, F., and Moore, A. U. Heart rate, respiration, audiogenic seizures. 201.
Marron, T. U. Vit. A., liver, methylcholanthrene. 219.
Martin, G. J., and Ansbacher, S. Vit. B. complex, p-aminobenzoic acid. 118.
Martin, W. P. 181.
Master, A. M. 207.
Mecchi, E. 526.
Mellish, C. H. 79.
Merino, C., Doan, C. A., Woolpert, O. C., Schwab, J., and Saslaw, S. Virus, influenza, streptococcus, mixed infections. 563; 558, 560, 566.
Mezera, R. A., Broun, G. O., Muether, R. O., and LeGier, M. Virus, encephalitis, gastrointesinal inoculation. 357, 310.
Milhorat, A. T., Bartels, W. E., and Toscani, V. Quinine, metabolism, creatinuria. 540.
Miller, H. R., Krueger, E., Alper, J. M., and Spiegel, E. A. Blood urea, hypothalamic lesions. 478.
Mitchell, H. S. 259.
Mitchell, L. C. 229.
Mixner, J. P., and Turner, C. W. Mamma lobule-alveolar growth, temperature. 443.
Monroe, W. M. 646.
Moore, A. U. 201.
Moore, K. 323.
Morgan, H. R., and Upham, H. C. Leucocyte migration, *Eberthella typhosa* antigen. 114.
Mueller, J. H., and Hinton, J. Gonococcus and meningococcus isolation. 330.
Muether, R. O. 310, 357.
Muhrer, M. E. 217.

Mulligan, R. M. Blood, bone marrow, X-rays. 607.
Mulliken, B. 598.

Najjar, V. A., and Holt, L. E., Jr. Urine fluorescent subst., pyridine compd. ingestion. 413.
Nastuk, C. L. 151.
Nelson, J. B. Virus, variola, vaccinia, inoculation in embryonated eggs. 454.
Nelson, W. O. 33.
Neter, E. Sulfonamides, *M. lysodeikticus* lysozyme lysis, bacteriostasis. 106.
Newman, H., and Abramson, M. Intoxication, alcohol conc. 509.
Nielsen, E., and Elvehjem, C. A. Biotin, spectacle eye. 349.
Nunez, C. 271.
Nungester, W. J., and Kempf, A. H. Pneumococci strains. 631.
Nungester, W. J., and Lewis, S. R. Antibody detn., by "falling drop." 425.

Ogden, E. 505.
Orr, J. H. 535.
Ouyang, G. Sulfonamides, *Corynebacterium diphtheriæ*. 41.

Pack, G. T. 488.
Palmes, E. D. 353.
Pang, K. H. Typhus, rat mites. 266.
Pangborn, M. C. Phospholipid, serologically active. 484.
Patrick, H., Boucher, R. V., Dutcher, R. A., and Knandel, H. C. Biotin, dermatitis. 456.
Pearson, H. E., and Enders, J. F. Virus, influenza A, culture. 140, 143.
Pearson, P. B., and Burgin, C. J. Vit., pantothenic acid, Royal Jelly. 415.
Peluffo, C. A. Antigen, Vi, *Salmonella typhi*, stability. 340.
Peoples, S. A., and Carmichael, E. B. Metabolism, respiratory, delvinal sodium. 381.
Perkins, W. 372.
Phillips, R. A. 158.
Pierce, M. E., Kempf, J. E., and Soule, M. H. Virus, equine encephalomyelitis, dilution, antiserum. 604; 187.
Popper, H. L., and Sorter, H. H. Blood enzymes, pancreas duct ligation. 384.
Poth, E. J., and Knotts, F. L. Sulfathiazole, succinyl, gastrointestinal bacteriostasis. 129.

Rahn, H., and Rosendale, F. Pituitary melanophore secretion. 100.
Rake, G., Eaton, M. D., and Shaffer, M. F. Virus, psittacosis, meningopneumonitis, lymphogranuloma venereum—relations. 528; 318.
Rakieten, M. L., and Bornstein, S. Bacteriophage, *Salmonella* and *E. typhi*, antigens. 359.
Rammelkamp, C. H., and Jewell, M. L. Sulfonamides, *staphylococcus aureus*. 27, 147.
Rammelkamp, C. H., and Weinstein, L. Tyrothricin, hemolysis. 211.
Rapoport, S. 541.
Reed, G. E., and Orr, J. H. Gas gangrene-toxin production. 535.
Rhoads, C. P. 22, 488.
Richter, C. P. Andrenalectomy, carbohydrate appetite. 577.
Richter, C. P., and Clisby, K. H. Hair graying by phenylthiourea. 684.
Richards, R. K., Kueter, K., and Klatt, T. J. Barbiturate action, vit. C deficiency. 403.
Richards, R. K., Spruth, H. C., and Russell, M. K. Estrus prolongation by estrogen crystals. 400.
Richardson, L. R., and Hogan, A. G. Antiperosis complex. 459.
Riggs, L. A. Retina, electrical activity. 204.
Riley, R. L. 280.
Robbins, E. B. 676.
Rocha e Silva, M., and Dragstedt, C. A. Heparin, liberation by trypsin. 152.
Rosen, M. J. 575.
Rosenberg, B., Krichesky, B., and Benjamin, J. A. Gonadotropic hormone, prostate. 263.
Rosendale, F. 100.
Rovenstine, E. A. 669.
Rule, A. M. 388.
Russell, M. K. 400.

Sabin, A. B., Ward, R., Rapoport, S., and Guest, G. M. Virus, poliomyelitis, neuroinvasion, vit. D. 451.
Sahyun, M. Metabolism, casein hydrolysate. 14.
Salmon, A. A. 11.
Salmon, U. J., Geist, S. H., and Salmon, A. A. Pregnandiol excretion, androgen. 11.
Sapirstein, L. A., Ogden, E., and Southard, F. D., Jr. Renin-like subst., hemorrhage. 505.
Sarracino, J. B., and Soule, M. H. Virus, influenza, resistance, cold, fatigue, alcohol. 183, 188.
Saslaw, S. 558, 560, 563, 566.
Satterfield, G. H. 473.
Schaffer, N. K., Barker, S. B., Summerson, W. H., and Stander, H. J. Eclampsia, blood uric, lactic acids, acetone. 237.
Schales, O., Hoobler, S. W., and Haynes, F. W. Renin, cardiovascular effects. 720.
Scharf, G., and Lyons, W. R. Mamma, estrone. 86.
Schechtman, A. M., and Cannon, J. A. Proctodeum, differentiation. 628.
Schlossman, N. 93.
Schotte, O. E., Butler, E. G., and Hood, R. T. Blastemas, transplants, amputated nerveless limbs. 500.
Schultz, E. W. Poliomyelitis, estrogen. 135.
Schwab, J. L., Saslaw, S., Woolpert, O. C., Merino, C., and Doan, C. A. *Streptococcus hemolyticus*, group C. 560; 558, 563, 566.
Scott, K. G., and Lawrence, J. H. P*, blood. 155.
Sebrell, W. H. 228.
Segaloff, A., and Nelson, W. O. Estrogen assay. 33.
Selle, W. A. Age, respiration, heart action, spinal, pupillary reflexes. 417.
Selye, H., and Dosne, C. Shock, adrenalectomy, blood sugar, cortin. 417.
Selzer, A., and Friedman, M. Hypertension, splanchnicectomy, renal blood flow. 429.
Shaffer, M. F. 528.
Shwartzman, G., Bender, M. B., and Wachtel, E. Iris, miotic action of phosphatides. 267; 612.
Sia, R. H. P. 109.
Simpson, M. E. 333, 634, 723.
Slaughter, D., Johnson, T. U., and Gales, J. W. Carnosine, muscle. 95.
Snyder, F. F., and Lim, K. T. Labor, morphine 197, 199.
Sobel, A. E., and Gottfried, S. P. Vit. D, antirachitic subst. from cholesteryl SO_4. 518.
Sommers, S. C. 639.
Sonnenblick, B. P., and Henshaw, P. S. Germ cell, X-ray induced dominant lethals. 74.

Sorter, H. H. 384.
Soule, M. H. 183, 187, 188, 604.
Southard, F. D., Jr. 505.
Spiegel, E. A. 478.
Spitzer, E. H., Coombes, A. I., Elvehjem, C. A., and Wisnicky, W. Vit. B, inactivation. 376.
Spruth, H. C. 400.
Stander, H. J. 237.
Stanley, A. J. 50.
Starbuck, E. B. 19.
Steinglass, P., Gordon, A. S., and Charipper, H. A. Castration, sex hormones, rat blood. 169.
Stern, K., and Dancey, T. E. Cerebrospinal fluid, D_2O. 619.
Stevens, J. E., Taliaferro, I., and Haag, H. B. Sulfathiazole, alimentary pleural, peritoneal absorption. 223.
Stevenson, E. S. 431.
Strauss, H. 674.
Strickler, H. S., Walton, M. E., and Wilson, D. A. Pregnandiol, bull urine. 37.
Sulkin, N. M. 624.
Sullivan, M. X. 353.
Summerson, W. H. 237.
Sweeney, H. M. 670.

Takacs, W. S. 637.
Talbot, J. C. 181.
Taliaferro, I. 223.
Taylor, F. A. Anthracosis pigment. 70.
Taylor, J. D. 302.
Tedeschi, C., and Schlossman, N. Tissue reactivity, bacterial filtrates. 93.
Terry, R. Vit. E deficiency, paralysis, Vit. B. 34.
Thatcher, J. S. 60.
Thomas, C. B. Hypertension, moderator nerves, kidney. 24.
Thompson, S. A., and Birnbaum, G. L. Resuscitation, asphyxial. 203.
Tischer, L. A. 637.
Tobin, C. E. Hormone, thyrotropic, pregnancy. 592.
Toomey, J. A., Takacs, W. S., and Tischer, L. A. Virus, poliomyelitis, flies. 637.
Toscani, V. 540.
Troescher-Elam, E., and Evans, H. M. Vit. B. factors. 549.
Tsao, S. N. Serum, bactericidal, estrogen. 38.
Turner, C. W. 439, 443.
Turner, J. C., and Mulliken, B. Oil, subcutaneous, disappearance. 598.
Tytell, A. A., and Kersten, H. Urease, catalase, soft X-rays. 521.

Upham, H. C. 114.

VanDyke, H. B. 368.
Van Herick, W. 247.
Van Wagenen, G., Himwich, H. E., and Catchpole, H. R. Milk, monkey, composition. 133.
Vargas, L. 271, 464, 467, 584.
Virtue, R. W. 326.
VonGlahn, W. C. 229.
Wachtel, E. 267.
Wakerlin, G. E. 513.
Walker, D. W. Streptococci, hemolytic, slide agglutination. 338.
Walker, H. A., and VanDyke, H. B. Malaria, sulfonamides. 368.
Walker, S. M., and Stanley, A. J. Lactation, stilbestrol. 50.
Walton, M. E. 37.
Wangerin, D. M. 726, 728.
Ward, R. 451.
Ward, T. G., and Starbuck, E. B. Growth, nicotinic acid, cysteine. 19.
Wartman, W. B. 274.
Waters, L. L. 304.
Weber, C. J., Lalich, J. J., and Major, R. H. Sulfapyridine, reduction of nitro derivative *in vivo*. 616.
Weil, A., and Heilbrunn, G. Sclerosis, disseminated. 233.
Weinstein, L., and Rammelkamp, C. H. Gramicidin by mouth. 147, 211.
Weiss, P. Melanin formation, thalamus deplants. 343.
Weiss, P., and Brown, P. F. Poliomyelitis, electromyographic studies. 284.
Weld, J. T. VonGlahn, W. C., and Mitchell, L. C. Liver cell inclusion, expl. 229.
Wells, W. F., and Henle, W. Virus, influenza, inoculation. 298.
Welt, L. G. Trichinosis, urinary trichina antigen. 587.
Werch, S. C., and Ivy, A. C. Galacturonic acid, absorption. 9.
Wertz, A. W., and Mitchell, H. S. Thiamin, pyrimidine in adults. 259.
White, A., and Stevenson, E. S. Cystine, rats, iodoacetic acid. 431; 590.
Wicks, L. F. Sex, proteinuria. 395.
Wilhelmi, A. E. 53.
Wilson, D. A. 37.
Winchester, C. F., and McKenzie, F. F. Sperm respiration, pH. 654; cell conc. 648.
Wisnicky, W. 376.
Wiswell, O. B. 278.
Witenberg, E. G. 302.
Woodhall, B. 555.
Woolpert, O. C., Schwab, J. L., Saslaw, S., Merino, C., and Doan, C. A. Virus, influenza A, respiratory infections. 558; 560, 563, 566.
Wulff, V. J. 656, 660.
Wyss, O. Sulfonamide, mode of action. 122.

Yarmush, I. G. 280.
Yen, C. H., and Chang, K. P. S. Typhoid, vi-phage protection. 243.
Young, N. F. 22.
Youngstrom, K. A., Woodhall, B., and Graves, R. W. Acetylcholine esterase, brain tumors. 555.

Zahl, P. A., and Waters, L. L. Tumors, dye localization. 304.
Zamcheck, N. 503.
Zellat, J. 544.
Zubin, J., and Barrera, S. E. Memory, electric convulsive therapy. 596.
Zucker, M. B. 372.

SUBJECT INDEX

(The numerals indicate the page.)

Acetylcholine esterase, brain tumors, 555.
Adrenalectomy, carbohydrate appetite, 577.
  hair growth, thyroid, 120.
Age, respiration, heart action, spinal, pupillary reflexes, 417.
Altitudes, infant, adult survival, 446.
Anesthetics, physical properties, 461.
Angiotonin, renal extracts, 372.
Anoxia, vascular response, 214.
Anthracosis pigment, 70.
Antibody detn., "falling drop," 425
Antigen, Vi, *Salmonella typhi* stability, 340.
Antitumoral action steroid hormones, 271.
Antrum cyst fluid, compn., 191.

Bacteriophage, *Salmonella* and *E. typhi* antigens, 359.
Barbiturate action, vit. C deficiency, 403.
Blastemas, transplants, amputated nerveless limbs, 500.
Blood, bone marrow, X-rays, 607.
  clotting globulin, 730.
  diastase distribution, 575.
  enzymes, pancreas duct ligation, 384.
  erythrocyte sedimentation rate, acetyl salicylic acid, rheumatic fever, 69.
  lipids, fasting, 411.
  pressure regulation, kidney, 513.
  serum antibodies, str. hemolytic, 56.
    antigonadotropic, 79.
    bactericidal, estrogen, 38.
  urea, hypothalamic lesions, 478.
  vessels, acetylcholine sensitization, 326.
Bone fractures, low Ca diet, 256.
Brain, bacterial filtrate reactions, 612.

Cancer, mamma, pseudopregnancy, 486.
Carbon tetrachloride poisoning protection by sulfonamides, 361.
Carbonic anhydrase, renal, 53.
Carnosine, muscle, 95.
Castration, sex hormones, rat blood, 169.
Catalase, soft X-rays, 521.
Cecitis, ulcerative, sulfaguanidine, 363.
Cerebrospinal fluid, D_2O, 619.
Chlorophyll, visceral accumulation and elimination, 438.
Cystine, bis-p-aminobenzoyl, 410.
  rats, iodoacetic acid, 431.

Digitalis, muscular weakness, 5.

Eclampsia, blood uric, lactic acids, acetone, 237.
Electrocardiography, fetal, 646.
Electroencephalogram, hyperventilation, age, 674
Embryo formation inhibition, X-rays, 624.
Erythroblastosis fetalis, pathogenesis, 126.
Estrus prolongation by estrogen crystals, 400.

Galacturonic acid, absorption, 9.
Gas gangrene-toxin production, 535.
  sampling inspired air, 346.
Gastric acidity, bone resorption, 98.
  secretion, histamine antagonist, 65.
Germ cell, X-ray induced dominant lethals, 74.
Glucosides, cardiac, 676.
Glucuronic acid, liver, butter yellow, 22.
Gonococcus, meningococcus isolation, 330.
  sulfathiazole, 476.
Gramicidin by mouth, 147.
Growth, lactation, dietary supplements, 692.
  nicotinic acid, cysteine, 19.

Hair graying by phenylthiourea, 684.
  growth, adrenalectomy, 120.
Heart, coronary occlusion, cardiac output, 207.
  rate, respiration, audiogenic seizures, 201.
  ventricular filling, 131.
Hemophilia-like disease, 217.
Heparin, liberation by trypsin, 152.
Hormone, androgens in placenta, 314.
  assay, 33.
    hemophilia, 167.
    tumor, 467.
    age, 584.
  estrogen absorption, pellets, 464.
  gonadotrophins, duality, 723.
    prostate, 263.
  lactogenic, implantation hypophysectomy, 315.
    mamma, stilbestrol, 439.
    vaginal opening, 634.
  pregnandiol, bull urine, 37.
    excretion, androgen, 11.
  sex, rat blood, 169.
  sodium retaining subst., assay, 60.
  thyrotropic, pregnancy, 592.
Hypertensin, amine-oxidase, tyrosinase action, 392.
Hypertension, moderator nerves, kidney, 24.
  pulse pressure, arteriovenous O_2, 492.
  splanchnicectomy, renal blood flow, 429.
Hyperthyroidism, bone Ca, 448.

Implantation, hypophysectomy, lactogenic hormone, 315.
Intestinal loop closure, 326.
Intoxication, alcohol conc., 509.
Iris, miotic action of phosphatides, 267.

Labor, morphine, 199.
Lactation, stilbestrol, 50.
Leucocyte migration, *Eberthella typhosa* antigen, 114.
Liver cell inclusion, expl., 229.
  cirrhosis, dietary, expl., prevention, 228.
  lipids, dinitrophenol, 302.
  vit. D, 274.
Luminescence, sulfonamide inhibition, 323.
Lymph flow, X-ray, 471.
Lymphogranuloma venereum, sulfonamides, 318.
Lysine deficiency, N balance, 728.

Malaria, pption. tests, 620.
  sulfonamides, 368.
Mamma, estrone, 86.
  growth, hypophysectomy, estrogen, prolactin, 590.
  lobule-alveolar growths, temperature, 443.
  progesterone, 83.
Mannitol hexanitrate, pharmacology, 72.
Melanin formation, thalamus deplants, 343.
Memory, electric convulsive therapy, 596.
Meningococcus isolation, 330.
Metabolism, casein hydrolysate, 14.
  head, Na pentobarbital, metrazol, 670.
  liver, body size, 419.
  respiratory, delvinal sodium, 381.
  tissue, triturus toxin, 423.
Micromanipulator electrode, 427.
Milk, monkey, composition, 133.
Myocarditis, sulfonamides, 1.

Nerve, electrical rectification, 293.
  optic, section, recovery pupillary function, 447.
  roots, spinal, skin temperature, 158.

Oil, subcutaneous, disappearance, 598.
Osteotrophy, chemotherapeutic agent, 672.

P*, blood, 155.
  disappearance, heart blood, 151.
Pancreas insulin, estrogen, 333.
Paraldehyde, toxicity, 669.
Perosis preventing complex, 459.
Phospholipid, serologically active, 484.
  sol'y, 195.
Phosphorylase, calcifying cartilage, 687.
Pituitary melanophore secretion, 100.
Pneumococci, specific sol. subst., 245.
  strains, 631.
Poliomyelitis, electromyographic studies, 284.
  estrogen, 135.
Pregnancy, ovariectomy, 221.
Proctodeum, differentiation, 628.

Quinine metabolism, creatinuria, 540.

Radiosensitivity, muscular and ciliary movement, 150.
Raynaud's disease, lung vol., cold, 503.
Relapsing fever, expl., immunity, 48.
Renal extracts angiotonin, tyramine, 372.
Renin, cardiovascular effects, 720.
  -like subst., hemorrhage, 505.
Respiration, O_2 breathing, N elimination, body size, 280.
Resuscitation, asphyxial, 203.
Retina, electrical activity, 204.
  pigment distribution, diurnal rhythm, 656.
  flicker response, 660.

Salmonella illinois, 240.
Sclerosis, disseminated, 233.
Scopolamine, fetus, 197.
Sensitization, heredity, 639.
Sex, proteinuria, 395.
  behavior, hormones, 667.
    intersexual fowl, 665.
Shock, adrenalectomy, blood sugar, cortin, 532.
Smoking, respiration, circulation, 495.
Sp. gallinarum, cultivation, 103.
Sperm respiration, cell conc., 648.
  iodoacetate, malonate, 570.
  pH, 654.
Streptococcus hemolyticus, group C, 560.
  reinoculation, 566.
  slide agglutination, 338.
Sulfapyridine, reduction of nitro derivative *in vivo*, 616.
Sulfathiazole, alimentary pleural, peritoneal absorption, 223.
  succinyl, gastrointestinal bacteriostasis, 129.
Sulfonamide, *B. diphtheriae*, 390.
  B. typhosus, 388.
  Corynebacterium diphtheriae, 41.
  expl. vaccinia, 138.
  M. lysodeikticus lysozyme lysis, 106.
  mode of action, 122.
  pyrimidine derivatives, 680.
  Staphylococcus aureus, 27.
  streptococci, 109.

Testis atrophy, X-rays, 44.
Tetanus toxin, anemia, 717.
Tissue reactivity, bacterial filtrates, 93.
Trichinosis, urinary trichina antigen, 587.
Trichomonas vaginalis, estrogens, 516.
Trypanosoma equiperdum cultivation, 90.
Tryptophane, chick needs, 526.
  deficiency, N balance, 726.
Tuberculosis, chlorin-e-rhodin-g, 435.
Tumor, artificial estrogens, 467.
  dye localization, 304.
  expl., food tars, 226.
  X-rays, neutrons, 251.
Typhoid, vi-phage protection, 243.
Typhus, rat mites, 266.
Tyrothricin, hemolysis, 211.

Urease, catalase, soft X-rays, 521.
Urine fluorescent subst., pyridine compd. ingestion, 413.

Vaccinia, sulfonamides, 138.
Virus, encephalitis, equine, antibodies, 247.
  dilution, antiserum, 604.
  gastrointestinal inoculation, 357.
  immunology, 310.
  influenza A, culture, 140.
  propylene glycol aerosol, 544.
  protamine pption., 481.
  resistance, 143.
  respiratory infections, 558.
  B, 177.
  inactivation of nasal secretion, 116.
  inoculation, 298.
  rat susceptibility, 181.
  resistance, cold, fatigue, alcohol, 183.
  sepn., feces, 188.
  streptococcus, mixed infections, 563.
  latent, immunity, 568.
  poliomyelitis, convalescent serum, 287.
  flies, 637.
  insulin, neurone injury, 187.
  neuroinvasion, vit. D, 451.
  pH, "Enteric organism filtrate", 579.
  psittacosis, meningopneumonitis, lymphogranuloma venereum—relations, 528.
  tobacco mosaic, cystine, 353.
  necrosis, sepn., 163.
  variola, vaccinia, inoculation in embryonated eggs, 454.
Vitamin A, liver, cancer, 488.
  methylcholanthrene, 219.
  B anti-perosis complex, 459.
  complex, p aminobenzoic acid, 118.
  factors, 549.
  inactivation, 376.
  biotin, dermatitis, 456.
  scaly dermatosis, 3.
  spectacle eye, 349.
  C deficiency, leg weakness, 473.
  D, antirachitic subst. from cholesteryl SO_4, 518.
  E, creatinuria, muscular dystrophy, 642.
  deficiency, paralysis, Vit. B, 34.
  K, hemorrhage control in *Eimeria tenella*, 278.
  nicotinuric acid, canine blacktongue, 355.
  pantothenic acid deficiency, H_2O metabolism, porphyrin, 112.
  Royal Jelly, 415.
  thiamin, pyrimidine in adults, 259.

All preliminary manuscripts are indicated by the letter P after the number of the article

INDEX

ABRAMSON, D. I., LANDT, H., and BENJAMIN, J. E. Peripheral Vascular Responses to General Anoxia 214
AEBERSOLD, P. C., 251.
ALTURE-WERBER, E. Cultivation of *Trypanosoma equiperdum* in the Yolk-Sac of Developing Chick Embryo........ 90
ANSBACHER, S., and LANDY, M. Biotin and Scaly Dermatosis of the Chick.... 3
ANSBACHER, S., 118.
ARMSTRONG, W. D., 98.
ATKINSON, W. B., 112.
AXELROD, D., AEBERSOLD, P. C., and LAWRENCE, J. H. Comparative Effects of Neutrons and X-Rays on Three Tumors Irradiated *in vitro* 251
BACK, A., 150.
BALDWIN, F. M., WISWELL, O. B., and JANKIEWICZ, H. A. Hemorrhage Control in *Eimeria tenella* Infected Chicks when Protected by Anti-Hemorrhagic Factor, Vitamin K................ 278
BARACH, A. L., 346.
BARKER, S. B., 237.
BECK, M. D., 177.
BENDER, M. B., 267.
BENJAMIN, J. A., 263.
BENJAMIN, J. E., 214.
BIGG, E. Influence of Specific Soluble Substance on Course of Experimental Pneumococcus Pneumonia 245
BING, R. J., ZUCKER, M. B., and PERKINS, W. Comparison between Destruction of Angiotonin, Hydroxytyramine and Tyramine by Renal Extracts 372
BIRCH, C. L. Assay of Estrogenic Hormone in Hemophilic and Normal Male Urine........ 167
BIRNBAUM, G. L., 203.
BLOOMFIELD, A. L., and LEW, W. Prophylactic Effect of Sulfaguanidine Against Ulcerative Cecitis in Rats 363
BOGART, R., 217.
BORNSTEIN, S., 359.
BRECHNER, J., and ARMSTRONG, W. D. Relation of Gastric Acidity to Alveolar Bone Resorption 98
BRIGHTMAN, I. J., 116.
BROUN, G. O., LE GIER, M., MEZERA, R. A., and MUETHER, R. O. Immunological Reaction of the Hamster to Inoculations with the Virus of St. Louis Encephalitis 310
BROUN, G. O., 357.
BROWN, H., 138.
BROWN, P. F., 284.
BRUNER, D. W., 240.
BUTCHER, E. O. Accelerated Hair Growth in the Rat after Adrenalectomy Cannot be Attributed to the Thyroid 120
CATCHPOLE, H. R., 133.
CHALECKI, W. E., WITENBERG, E. G., TAYLOR, J. D., and HODGE, H. C. Influence of 2-4 Dinitrophenol on Liver Lipids of the White Mouse 302

CHANG, K. P. S., 243.		
CHARIPPER, H. A., 169.		
CHEN, Y. P.	Immunity and Experimental Relapsing Fever in the Chinese Hamster *(Cricetulus griseus)*	48
CHICKERING, O., 65.		
COHEN, S. S.	Separation of Tobacco Necrosis Virus and Tobacco Mosaic Virus	163
COLE, K. S., 293.		
COLE, W. H., and NASTUK, C. L.	Disappearance of Radioactive Phosphorus from Heart Blood of *Limulus polyphemus*	151
CONRAT, H. L. F., HERRING, V. V., SIMPSON, M. E., and EVANS, H. M.	Mechanism of Action of Estrogens on Insulin Content of the Rat's Pancreas	333
COOMBES, A. I., 376.		
COURNAND, A., YARMUSH, I. G., and RILEY, R. L.	Influence of Body Size on Gaseous Nitrogen Elimination During High Oxygen Breathing	280
CUNNINGHAM, B., and KUHN, H. H.	Presence of Androgens in the Placenta	314
CUTULY, E.	Implantation Following Mating in Hypophysectomized Rats Injected with Lactogenic Hormone	315
DAFT, F. S., SEBRELL, W. H., and LILLIE, R. D.	Production and Apparent Prevention of a Dietary Liver Cirrhosis in Rats	228
DANN, W. J., and HANDLER, P.	Inactivity of Nicotinuric Acid in Canine Blacktongue	355
DAVENPORT, H. W., and WILHELMI, A. E.	Renal Carbonic Anhydrase	53
DIEFENDORF, H. W.	A Method for Detecting in Human Serum Protective Bodies Against Hemolytic Streptococci	56
DOSTER-VIRTUE, M. E., and VIRTUE, R. W.	An Improved Closure for the Doster-Virtue Intestinal Loop	326
DRAGSTEDT, C. A., 152.		
DREISBACH, R., and SNYDER, F. F.	Effect of Scopolamine on the Fetus	197
EATON, M. D., and BECK, M. D.	A New Strain of Virus of Influenza B Isolated During an Epidemic in California	177
EATON, M. D., MARTIN, W. P., and TALBOT, J. C.	Susceptibility of Kangaroo Rats to Strains of Human Influenza Virus	181
ECKMAN, M., and BARACH, A. L.	A Method for Obtaining Gas Samples of the Total Inspired Air	346
EDWARDS, P. R., and BRUNER, D. W.	A New Salmonella Type, *Salmonella illinois*	240
EICHELBERGER, L., and LINDSAY, J. R.	Chemical Composition of Fluids from Benign Cysts of the Antrum	191
ELVEHJEM, C. A., 349, 376.		
ENDERS, J. F., 140.		
ENDERS, J. R., and PEARSON, H. E.	Resistance of Chicks to Infection with Influenza A Virus	143
EVANS, H. M., 333.		
FIGGE, F. H. J., and ATKINSON, W. B.	Relation of Water Metabolism to Porphyrin Incrustations in Pantothenic Acid-Deficient Rats	112
FORBES, J. C., 361.		
FRANCIS, T., Jr., and BRIGHTMAN, I. J.	Virus-Inactivating Capacity of Nasal Secretions in the Acute and Convalescent Stages of Influenza	116
FRAZIER, C. N., 44.		

FRENCH, A. J. Interstitial Myocarditis in Mice and Rats Produced by Neoprontosil, Sulfanilamide, Sodium Sulfapyridine and Sodium Sulfathiazole 1
FREY, C. N., 256.
GALES, J. W., 95.
GEIST, S. H., 11.
GOLDHABER, G., and BACK, A. Studies on Radiosensitivity of Animal Cell *in vitro.* I. Radiosensitivity of Muscular and Ciliary Movement 150
GORDON, A. S., 169.
GRAY, S. W. Rôle of the Auricles in Ventricular Filling of the Tortoise Heart 131
GRISHMAN, A., and MASTER, A. M. Cardiac Output in Coronary Occlusion Studied by the Sezler-Boeger Physical Method 207
GUTTMAN, R., and COLE, K. S. Electrical Rectification in Single Nerve Fibers 293
HAAG, H. B., 223.
HANDLER, P., 355.
HARE, K., 158.
HARTMAN, C. G. Non-Effect of Ovariectomy on the Twenty-fifth Day of Pregnancy in the Rhesus Monkey 221
HARTMAN, F. A., LEWIS, L. A., and THATCHER, J. S. Assay of Sodium-Retaining Substances 60
HEILBRUNN, G., 233.
HENLE, W., 298.
HENSHAW, P. S., 74.
HEPPEL, L. A. A Pharmacological Study of Mannitol Hexanitrate 72
HERRING, V. V., 333.
HESS, W. C., SULLIVAN, M. X., and PALMES, E. D. Colorimetric Determination of Cystine in Tobacco Mosaic Virus Protein 353
HEYMANN, W., and WARTMAN, W. B. Effect of Vitamin D on Binucleated Cells of the Liver 274
HIMWICH, H. E., 133.
HINSEY, J. C., HARE, K., and PHILLIPS, R. A. Regulation of Skin Temperatures after Lesions of Spinal Nerve Roots 158
HINTON, J., 330.
HOAGLAND, H. Sensitization of Blood Vessels to Acetylcholine by Sympathetic Denervation 326
HODGE, H. C., 302.
HOGAN, A. G., MUHRER, M. E., and BOGART, R. A Hemophilia-Like Disease in Swine 217
HOOKER, S. P., 69.
HOWITT, B. F., and VAN HERICK, W. Neutralizing Antibodies Against St. Louis and Western Equine Encephalitic Viruses in Horses and Fowl 247
HU, C. H., 226.
HU, C. K., and FRAZIER, C. N. Atrophy of Germinal Epithelium of the Rabbit's Testis Induced by Roentgen Rays 44
HUANG, C. H., and SIA, R. H. P. Effects of Sulfanilamide and Sulfapyridine upon Experimental Streptococcal Infections 109
IVY, A. C., 9.
JANKIEWICZ, H. A., 278.
JEWELL, M. L., 27.

JOHNSON, F. H., and MOORE, K.	Sulfonamide Inhibition of Bacterial Luminescence	323
JOHNSON, T. U., 95.		
JONES, H. P., RAKE, G., and MC KEE, C. M.	Chemotherapy of Lymphogranuloma Venereum with Sulfonamide Drugs	318
KAPLAN, D., 103.		
KATZIN, E. M., 126.		
KEMPF, J. E., PIERCE, M. E., and SOULE, M. H.	Failure to Produce Neuronal Injury and Necrosis with Poliomyelitis Virus in Rabbits During Insulin Hypoglycemia	187
KENSLER, C. J., YOUNG, N. F., and RHOADS, C. P.	Glucuronic Acid Production *in vitro* by Liver Slices from Rats Fed Dimethylaminoazobenzene (Butter Yellow)	22
KLIGLER, I. J., and KAPLAN, D.	Studies on the Cultivation of *Sp. gallinarum*	103
KNOTTS, F. L., 129.		
KOLMER, J. A., and BROWN, H.	Failure of Sulfanilamide in Treatment of Experimental Vaccinia of Rabbits	138
KRAMER, S. D.	Protection in White Mice with Human Convalescent Serum Against Infection with Poliomyelitis Virus (Armstrong Strain)	287
KRICHESKY, B., 263.		
KUHN, H. H., 314.		
KUPPERMAN, H. S., MELLISH, C. H., and MC SHAN, W. H.	Specificity of Fowl and Mammalian Antigonadotropic Sera	79
LADUE, J. S.	Generalized Muscular Weakness as a Toxic Reaction to Digitalis	5
LANDT, H., 214.		
LANDY, M., 3.		
LAWRENCE, J. H., 155, 251.		
LEACH, B. E., and FORBES, J. C.	Sulfonamide Drugs as Protective Agents Against Carbon Tetrachloride Poisoning	361
LE GIER, M., 310, 357.		
LEVINE, P., and KATZIN, E. M.	Pathogenesis of Erythroblastosis fetalis: Absence of the Rh Factor from Saliva	126
LEW, W., 363.		
LEWIS, L. A., 60.		
LICHTY, J. A., Jr., and HOOKER, S. P.	Effect of Acetyl Salicylic Acid on Sedimentation Rate of Erythrocytes in Rheumatic Fever	69
LIGHT, R. F., and FREY, C. N.	Bone Fracture Due to Low Calcium Diets	256
LILLIE, R. D., 228.		
LIM, K. T., 199.		
LINDSAY, J. R., 191.		
LIPSCHUTZ, A., VARGAS, L., and NUNEZ, C.	Comparative Antitumoral Action of Desoxycorticosterone Acetate and Testosterone Propionate	271
LIU, Y., and HU, C. H.	Experimental Production of Tumors by Tars from Various Foods	226
LOEW, E. R., and CHICKERING, O.	Gastric Secretion in Dogs Treated with Histamine Antagonist, Thymoxyethyldiethylamine	65
LYONS, W. R., and MC GINTY, D. A.	Effects of Estrone and Progesterone on Male Rabbit Mammary Glands. I. Varying Doses of Progesterone	83
LYONS, W. R., 86.		

MC EWEN, H. D., and MAC LACHLAN, P. L.	Effect of Digitonin on Solubility of Phospholipids	195
MC GINTY, D. A., 83.		
MC KEE, C. M., 318.		
MC SHAN, W. H., 79.		
MAC LACHLAN, P. L., 195.		
MARCUSE, F., and MOORE, A. U.	Heart Rate and Respiration Preceding and Following Audiogenic Seizures in the White Rat	201
MARRON, T. U.	Return of Hepatic Vitamin A in Rats after Depletion by Methylcholanthrene	219
MARTIN, G. J., and ANSBACHER, S.	Rôle of Para-Aminobenzoic Acid in Vitamin B-Complex Studies with Mice	118
MARTIN, W. P., 181.		
MASTER, A. M., 207.		
MELLISH, C. H., 79.		
MEZERA, R. A., BROUN, G. O., MUETHER, R. O., and LE GIER, M.	Intragastric and Intraintestinal Inoculations with St. Louis Encephalitis Virus	357
MEZERA, R. A., 310.		
MITCHELL, H. S., 259.		
MITCHELL, L. C., 229.		
MOORE, A. U., 201.		
MOORE, K., 323.		
MORGAN, H. R., and UPHAM, H. C.	Effect of Antigenic Material from *Eberthella typhosa* upon Migration of Guinea Pig Leucocytes	114
MUELLER, J. H., and HINTON, J.	A Protein-Free Medium for Primary Isolation of the Gonococcus and Meningococcus	330
MUETHER, R. O., 310, 357.		
MUHRER, M. E., 217.		
NASTUK, C. L., 151.		
NELSON, W. O., 33.		
NETER, E.	Action of Sulfamido Compounds upon *M. lysodeikticus* and Lytic and Bactericidal Activities of Lysozyme	106
NIELSEN, E., and ELVEHJEM, C. A.	Cure of Spectacle Eye Condition in Rats with Biotin Concentrates	349
NUNEZ, C., 271.		
OUYANG, G.	*In vitro* effect of Sulfanilamide, Sulfapyridine and Sulfathiazole on *Corynebacterium diphtheriae*	41
PALMES, E. D., 353.		
PANG, K. H.	Isolation of Typhus Rickettsia from Rat Mites During Epidemic in an Orphanage	266
PEARSON, H. E., and ENDERS, J. F.	Cultivation of Influenza A Virus in Roller Tubes	140
PEARSON, H. E., 143.		
PELUFFO, C. A.	Stability of Vi Antigen of *Salmonella typhi*	340
PERKINS, W., 372.		
PHILLIPS, R. A., 158.		
PIERCE, M. E., 187.		
POTH, E. J., and KNOTTS, F. L.	Succinyl Sulfathiazole, a New Bacteriostatic Agent Locally Active in the Gastrointestinal Tract	129

RAHN, H., and ROSENDALE, F. Diurnal Rhythm of Melanophore Hormone Secretion in the Anolis Pituitary 100

RAKE, G., 318.

RAKIETEN, M. L., and BORNSTEIN, S. Influence of Certain Bacteriophages on the H Antigens of *Salmonella poona* and *E. typhi*. 359

RAMMELKAMP, C. H., and JEWELL, M. L. Comparative Study of Effect of Sulfadiazine and Sulfathiazole on *Staphylococcus aureus* 27

RAMMELKAMP, C. H., and WEINSTEIN, L. Hemolytic Effect of Tyrothricin 211

RAMMELKAMP, C. H., 147.

RHOADS, C. P., 22.

RIGGS, L. A. Continuous and Reproducible Records of the Electrical Activity of the Human Retina 204

RILEY, R. L., 280.

ROCHA E SILVA, M., and DRAGSTEDT, C. A. Liberation of Heparin by Trypsin 152

ROSENBERG, B., KRICHESKY, B., and BENJAMIN, J. A. Response of Intraocular Prostatic Implants in Rabbits to Gonadotropic Hormone Administration 263

ROSENDALE, F., 100.

SAHYUN, M. Some Aspects of Metabolism Following Parenteral Administration of Casein Hydrolysate 14

SALMON, A. A., 11.

SALMON, U. J., GEIST, S. H., and SALMON, A. A. Effect of Androgen Administration upon Pregnandiol Excretion in Cyclical Women 11

SARRACINO, J. B., and SOULE, M. H. Effect of Heat, Cold, Fatigue and Alcohol on Resistance of Mice to Human Influenza Virus 183

Isolation of Virus from Feces of Mice Receiving Intranasal Inoculations of Epidemic Influenza Virus 188

SCHAFFER, N. K., BARKER, S. B., SUMMERSON, W. H., and STANDER, H. J. Relation of Blood Lactic Acid and Acetone Bodies to Uric Acid in Pre-Eclampsia and Eclampsia 237

SCHARF, G., and LYONS, W. R. Effects of Estrone and Progesterone on Male Rabbit Mammary Glands. II. Varying Doses of Estrone 86

SCHLOSSMAN, N., 93.

SCHULTZ, E. W. Estrogen Intranasally in Experimental Poliomyelitis 135

SCOTT, K. G., and LAWRENCE, J. H. Effect of Radio-Phosphorus on Blood of Monkeys 155

SEBRELL, W. H., 228.

SEGALOFF, A., and NELSON, W. O. Absorption and Metabolism of Estrogens. I. Estrogen Assay by Intrasplenic Injection 33

SHWARTZMAN, G., BENDER, M. B. and WACHTEL, E. Miotic Action of Lecithin and Cephalin on Denervated Iris of Cat, Monkey, Dog and Rabbit 267

SIA, R. H. P., 109.

SIMPSON, M. E., 333.

SLAUGHTER, D., JOHNSON, T. U., and GALES, J. W. Smooth Muscle Effects of Carnosine 95

SNYDER, F. F., and LIM, K. T. Effect of Morphine on Labor 199

SNYDER, F. F., 197.

SONNENBLICK, B. P., and HENSHAW, P. S.	Influence on Development of Certain Dominant Lethals Induced by X-rays in Drosophila Germ Cells	74
SOULE, M. H., 183, 187, 188.		
SPITZER, E. H., COOMBES, A. I., ELVEHJEM, C. A., and WISNICKY, W.	Inactivation of Vitamin B_1 by Raw Fish	376
STANDER, H. J., 237.		
STANLEY, A. J., 50.		
STARBUCK, E. B., 19.		
STEINGLASS, P., GORDON, A. S., and CHARIPPER, H. A.	Effect of Castration and Sex Hormones on Blood of Rat	169
STEVENS, J. E., TALIAFERRO, I., and HAAG, H. B.	Absorption of Sulfathiazole from the Gastrointestinal Tract, Pleura and Peritoneum of Dogs	223
STRICKLER, H. S., WALTON, M. E., and WILSON, D. A.	Failure to Find Sodium Pregnandiol Glucuronidate in Bull's Urine	37
SULLIVAN, M. X., 353.		
SUMMERSON, W. H., 237.		
TALBOT, J. C., 181.		
TALIAFERRO, I., 223.		
TAYLOR, F. A.	Pigment of Anthracosis	70
TAYLOR, J. D., 302.		
TEDESCHI, C., and SCHLOSSMAN, N.	Phenomenon of Local Tissue Reactivity to Bacterial Filtrates Elicited in Heart and Femoral Artery	93
TERRY, R.	Ineffectiveness of Thiamin Hydrochloride in Treating Late Weaning Paralysis of Vitamin E-deficient Rats	35
THATCHER, J. S., 60.		
THOMAS, C. B.	Experimental Hypertension from Section of Moderator Nerves: Relationship to Presence of Kidney Tissue	24
THOMPSON, S. A., and BIRNBAUM, G. L.	Phenomenon of Asphyxial Resuscitation. Resuscitation with Inert (Asphyxiating) Gases	203
TSAO, S. N.	Bactericidal Property of Blood Serum of Male Rabbits Treated with Urinary Estrogens	38
UPHAM, H. C., 114.		
VAN DYKE, H. B., 368.		
VAN HERICK, W., 247.		
VAN WAGENEN, G., HIMWICH, H. E., and CATCHPOLE, H. R.	Composition of the Milk of the Monkey *(M. mulatta)*	133
VARGAS, L., 271.		
VIRTUE, R. W., 326.		
VON GLAHN, W. C., 229.		
WACHTEL, E., 267.		
WALKER, D. W.	Application of the Technic of Slide Agglutination of Hemolytic Streptococci to Human Sera	338
WALKER, H. A., and VAN DYKE, H. B.	Control of Malaria Infection *(P. lophurae)* in Ducks by Sulfonamides	368
WALKER, S. M., and STANLEY, A. J.	Effect of Diethylstilbestrol Dipropionate on Mammary Development and Lactation	50
WALTON, M. E., 37.		

WARD, T. G., and STARBUCK, E. B. Enhancing Effect of Nicotinic Acid and Cysteine Hydrochloride on Growth of *Leptospira icterohaemorrhagiae* 19
WARTMAN, W. B., 274.
WATERS, L. L., 304.
WEIL, A., and HEILBRUNN, G. Demonstration of Myelolytic Substances in Disseminated Sclerosis 233
WEINSTEIN, L., and RAMMELKAMP, C. H. A Study of the Effect of Gramicidin Administered by the Oral Route 147
WEINSTEIN, L., 211.
WEISS, P. Melanin Formation by Deplanted Fragments of Thalamus in Amphibian Larvae 343
WEISS, P., and BROWN, P. F. Electromyographic Studies on Re-coördination of Leg Movements in Poliomyelitis Patients with Transposed Tendons 284
WELD, J. T., VON GLAHN, W. C., and MITCHELL, L. C. Production of Cytoplasmic Inclusions in Liver Cells of Rats Injected with Certain Proteins 229
WELLS, W. F., and HENLE, W. Quantitative Inoculation by Inhalation of Influenza Virus 298
WERCH, S. C., and IVY, A. C. Is Galacturonic Acid Absorbed by the Small and Large Intestine? 9
WERTZ, A. W., and MITCHELL, H. S. Thiamin and Pyrimidine Studies on Older Subjects 259
WILHELMI, A. E., 53.
WILSON, D. A., 37.
WISNICKY, W., 376.
WISWELL, O. B., 278.
WITENBERG, E. G., 302.
WYSS, O. Nature of Sulfanilamide Inhibition 122
YARMUSH, I. G., 280.
YEN, C. H., and CHANG, K. P. S. Protective Values of Various Types of Vi-Phage on Experimental Typhoid Infection in Mice 243
YOUNG, N. F., 22.
ZAHL, P. A., and WATERS, L. L. Localization of Colloidal Dyes in Animal Tumors 304
ZUCKER, M. B., 372.

All preliminary manuscripts are indicated by the letter P after the number of the article

INDEX

ABELS, J. C., GORHAM, A. T., PACK, G. T., and RHOADS, C. P.	Metabolic Studies in Patients with Gastrointestinal Cancer. Hepatic Concentrations of Vitamin A	488
ABRAMSON, M., 509.		
ALLISON, S. D., 476.		
ALMQUIST, H. J., and MECCHI, E.	Tryptophane Requirement of the Chick	526
ALPER, J. M., 478.		
ALTSCHULE, M. D., LINENTHAL, H., and ZAMCHECK, N.	Lung Volume and Pulmonary Dynamics in Raynaud's Disease. Effect of Exposure to Cold	503
ANÉ, J. N., and BURCH, G. E.	Effects of Roentgen Irradiation upon Linear Rate of Flow in Cutaneous Lymphatics of Humans	471
APPERLY, F. L., and CARY, M. K.	Relation of Arterial Pulse-Pressure to Arterio-Venous Oxygen Difference, Especially in Arterial Hypertension	492
BARTELS, W. E., 540.		
BELL, T. A., SATTERFIELD, G. H., and COOK, F. W.	"Leg-Weakness" in Laying Hens on an Ascorbic Acid-Free (Vitamin C) Diet	473
BENDER, M. B., 447.		
BOUCHER, R. V., 456.		
BRUZZONE, S., 467.		
BURCH, G. E., 471.		
BURGIN, C. J., 415.		
BUTLER, E. G., 500.		
CARMICHAEL, E. B., 381.		
CARPENTER, C. M., CHARLES, R., and ALLISON, S. D.	Effect of Gradually Increased Concentrations of Sulfathiazole on the Gonococcus *in vitro*	476
CARY, M. K., 492.		
CHAMBERS, L. A., and HENLE, W.	Precipitation of Active Influenza A Virus from Extra-embryonic Fluids by Protamine	481
CHARLES, R., 476.		
CONE, N. M., FORMAN, S. E., and KRANTZ, J. C., Jr.	Relationship Between Anesthetic Potency and Physical Properties	461
COOK, F. W., 473.		
CROXATTO, H., and CROXATTO, R.	Inhibitory Action of Amine-Oxidase and Tyrosinase upon Vasoconstrictor Effect of Hypertensin	392
CROXATTO, R., 392.		
CUNNINGHAM, R. W., 410.		
DOSNE, C., 532.		
DRILL, V. A.	Bone Calcium During Hyperthyroidism	448
DUTCHER, R. A., 456.		
EATON, M. D., 528.		
EGANA, E., 467.		
FAZEKAS, J. F., 446.		
FELLOWS, E. J., and CUNNINGHAM, R. W.	Bis-para-aminobenzoyl-l-cystine	410
FIELD, J., II, 423.		
FORMAN, S. E., 461.		

FRIEDMAN, M., 429.		
FUHRMAN, F. A., and FIELD, J., II.	Effect of Triturus Toxin on Respiration of Rat Tissue *in vitro*	423
FULTON, G. P.	A Silver-glass Micro-electrode for Use in a Micromanipulator	427
GORHAM, A. T., 488.		
GOTTFRIED, S. P., 518.		
GUEST, G. M., 451.		
HENDERSON, H. J., and LONG, E. R.	Effect of Chlorin-e-Rhodin-g on Experimental Tuberculosis	435
	Accumulation of Chlorophyll Pigments in Visceral Organs and Their Elimination	438
HENLE, W., and ZELLAT, J.	Effect of Propylene Glycol Aerosol on Air-Borne Virus of Influenza A	544
HENLE, W., 481.		
HERRLICH, H. C., FAZEKAS, J. F., and HIMWICH, H. E.	Survival of Infant and Adult Rats at High Altitudes	446
HIMWICH, H. E., 446.		
HOGAN, A. G., 459.		
HOLT, L. E., Jr., 413.		
HOOD, R. T., 500.		
HOUSE, R. M., and WAKERLIN, G. E.	Possible Rôle of the Kidney in Regulation of Normal Blood Pressure in the Dog	513
JOHNSON, G., 516.		
KERSTEN, H., 521.		
KLATT, T. J., 403.		
KLEIBER, M.	Body Size and Metabolism of Liver Slices *in vitro*	419
KNANDEL, H. C., 456.		
KOLMER, J. A.	Failure of Sulfonamide Compounds in Treatment of Experimental *B. diphtheriae (Corynebacterium diphtheriae)* Infections of Guinea Pigs	390
KOLMER, J. A., and RULE, A. M.	Sulfonamide Compounds in Treatment of Experimental *B. typhosus (Eberthella typhosus)* Infections of Rabbits	388
KRANTZ, J. C., Jr., 461.		
KRUEGER, E., 478.		
KUETER, K., 403.		
KUPFERBERG, A. B., and JOHNSON, G.	Physiology of Bacteria-Free Culture of *Trichomonas vaginalis.* Effect of Female Sex Hormones on Population	516
LAW, L. W.	Effect of Pseudopregnancy on Mammary Carcinoma Incidence in Mice of the A Stock	486
LEWIS, A. A., and TURNER, C. W.	Effect of Stilbestrol on Lactogenic Content of Pituitary and Mammary Glands of Female Rats	439
LEWIS, S. R., 425.		
LINENTHAL, H., 503.		
LIPSCHÜTZ, A., and VARGAS, L., Jr.	Absorption of Estradiol and its Esters from Subcutaneously Implanted Tablets in Guinea Pig	464
LIPSCHÜTZ, A., VARGAS, L., Jr., EGANA, E., and BRUZZONE, S.	Conjunctive Tumorigenesis Elicited by Different Artificial Estrogens	467
LONG, E. R., 435, 438.		

LOWENSTEIN, O., and BENDER, M. B. Recovery of Pupillary Function in Cut Optic Nerves of Cats and Monkeys ... 447
MAC LACHLAN, P. L. Increase of Blood Lipids of Fasted Mice ... 411
MAIN, R. J. Acute Effects of Smoking on Respiration and Circulation ... 495
MECCHI, E., 526.
MILHORAT, A. T., BARTELS, W. E., and TOSCANI, V. Effect of Quinine on Metabolism in Fasting Dogs and in Patients with Creatinuria ... 540
MILLER, H. R., KRUEGER, E., ALPER, J. M., and SPIEGEL, E. A. Changes in Blood Urea Following Hypothalamic Lesions ... 478
MIXNER, J. P., and TURNER, C. W. Influence of Environmental Temperature on Growth of Mammary Lobule-Alveolar System ... 443
NAJJAR, V. A., and HOLT, L. E., Jr. Effect of Ingestion of Various Pyridine Compounds on Excretion of Specific Fluorescent Substances in Urine ... 413
NELSON, J. B. Simultaneous Inoculation of Variola and Vaccinia Viruses in Embryonated Eggs ... 454
NEWMAN, H., and ABRAMSON, M. Relation of Alcohol Concentration to Intoxication ... 509
NUNGESTER, W. J., and LEWIS, S. R. Application of ''Falling Drop'' Measurement of Density to Quantitative Determination of Antibody in Immunological Reactions ... 425
OGDEN, E., 505.
ORR, J. H., 535.
PACK, G. T., 488.
PANGBORN, M. C. A New Serologically Active Phospholipid from Beef Heart ... 484
PATRICK, H., BOUCHER, R. V., DUTCHER, R. A., and KNANDEL, H. C. Biotin and Prevention of Dermatitis in Turkey Poults ... 456
PEARSON, P. B., and BURGIN, C. J. Pantothenic Acid Content of Royal Jelly ... 415
PEOPLES, S. A., and CARMICHAEL, E. B. Effect of Delvinal Sodium on Respiratory Metabolism of the Rhesus Monkey *(Macaca mulatta)* ... 381
POPPER, H. L., and SORTER, H. H. Blood Enzymes after Ligation of all Pancreatic Ducts ... 384
RAKE, G., EATON, M. D., and SHAFFER, M. F. Similarities and Possible Relationships among Viruses of Psittacosis, Meningopneumonitis, and Lymphogranuloma venereum ... 528
RAPOPORT, S., 451.
REED, G. B., and ORR, J. H. Gas Gangrene-Toxin Production in Gelatin-Thioglycollate Medium ... 535
RHOADS, C. P., 488.
RICHARDS, R. K., KUETER, K., and KLATT, T. J. Effect of Vitamin C Deficiency on Action of Different Types of Barbiturates ... 403
RICHARDS, R. K., SPRUTH, H. C., and RUSSELL, M. K. Prolongation of Estrus by Injection of Suspensions of Estrogen Crystals ... 400
RICHARDSON, L. R., and HOGAN, A. G. The Anti-Perosis Complex ... 459
RULE, A. M., 388.
RUSSELL, M. K., 400.
SABIN, A. B., WARD, R., RAPOPORT, S., and GUEST, G. M. Neuroinvasiveness of Poliomyelitis Virus in Relation to Vitamin D Nutrition ... 451
SAPIRSTEIN, L. A., OGDEN, E., and SOUTHARD, F. D., Jr. Renin-Like Substance in Blood after Hemorrhage ... 505

SATTERFIELD, G. H., 473.

SCHOTTE, O. E., BUTLER, E. G., and HOOD, R. T. Effects of Transplanted Blastemas on Amputated Nerveless Limbs of Urodele Larvae.... 500

SELLE, W. A. Influence of Age on Survival of Respiration, Spinal Reflexes, Pupillary Responses and Heart Action 417

SELYE, H., and DOSNE, C. Influence of Traumatic Shock on Blood Sugar of Adrenalectomized Rats Treated with Adrenal Cortical Extract 532

SELZER, A., and FRIEDMAN, M. Effect of Bilateral Splanchnicectomy upon Renal Blood Flow in Hypertension 429

SHAFFER, M. F., 528.

SOBEL, A. E., and GOTTFRIED, S. P. Formation of an Antirachitic Product from Cholesteryl Sulfates 518

SORTER, H. H., 384.

SOUTHARD, F. D., Jr., 505.

SPIEGEL, E. A., 478.

SPRUTH, H. C., 400.

STEVENSON, E. S., 431.

TOSCANI, V., 540.

TURNER, C. W., 439, 443.

TYTELL, A. A., and KERSTEN, H. Effects of Soft X-rays on Urease and Catalase 521

VARGAS, L., Jr., 464, 467.

WAKERLIN, G. E., 513.

WARD, R., 451.

WHITE, A., and STEVENSON, E. S. Effect of Oral Administration of Iodoacetic Acid on Cystine Content of Rats 431

WICKS, L. F. Sex and Proteinuria of Mice 395

ZAMCHECK, N., 503.

ZELLAT, J., 544.

All preliminary manuscripts are indicated by the letter P after the number of the article

INDEX

ALBANESE, A. A., HOLT, L. E., JR., BRUMBACK, J. E., JR., HAYES, M., KAJDI, D., and WANGERIN, D. M. Nitrogen Balance in Experimental Lysine Deficiency in Man 728
ALBANESE, A. A., 726.
ANDERSON, R. C., 676.
BALL, Z. B., and BARNES, R. H. Effect of Various Dietary Supplements on Growth and Lactation in the Albino Mouse 692
BARNES, R. H., 692.
BARRERA, S. E., 596.
BEARD, M., 566.
BENDIEN, W. M., and SOMMER, H. Purification of Paralytic Shellfish Poison by Filtration Through Active Charcoal 715
BERNHARD, A., and ROSEN, M. J. Distribution of Diastase in Serum, Plasma, Whole Blood and Red Blood Cells 575
BIRD, R. M., McSWAIN, B., KAUER, G. L., and GLENN, F. Clotting Action of Rabbit-Clotting Globulin 730
BLATTNER, R. J., and HEYS, F. M. Experimental Transmission of St. Louis Encephalitis to White Swiss Mice by *Dermacentor variabilis* 707
BROOKMAN, B. T., 670.
BRUMBACK, J. E., JR., 726, 728.
BURSTEIN, C. L., and ROVENSTINE, E. A. Toxicity of Intravenous Paraldehyde 669
CANNON, J. A., 628.
CHEN, K. K., ANDERSON, R. C., and ROBBINS, E. B. K-Strophanthin-β, K-Strophanthoside, Periplocymarin and Periplocin 676
CLISBY, K. H., 684.
DANCEY, T. E., 619.
DAVIS, D. E., and DOMM, L. V. Sexual Behavior of Hormonally Treated Domestic Fowl 667
DAVIS, D. E., 665.
DOAN, C. A., SASLAW, S., BEARD, M., WOOLPERT, O. C., SCHWAB, J. L., and MERINO, C. Reactions of Monkeys to Reinoculation with *Streptococcus hemolyticus* 566
DOAN, C. A., 558, 560, 563.
DOMM, L. V., and DAVIS, D. E. Sexual Behavior of Intersexual Domestic Fowl 665
DOMM, L. V., 667.
DULANEY, A. D., and HOUSE, V. Precipitative Tests in Malaria 620
EMERSON, G. A., and EMERSON, M. J. Mechanism of Effect of Epinephrine on Bioluminescence of the Firefly 700
EMERSON, M. J., 700.
ERCOLI, N. A Chemotherapeutic Agent with Osteotropic Properties 672
EVANS, H. M., 549, 634, 723.
EVANS, T. C., LEVIN, A. J., and SULKIN, N. M. Inhibition of Embryo Formation in Certain Nematodes by Roentgen Radiation 624
FARKAS, H., and KLIGLER, I. J. Production of Anemia by Tetanus Toxin 717
FELLOWS, E. J. Substituted Sulfanilamidopyrimidine 680

FRAENKEL-CONRAT, H., LI, C. H., SIMPSON, M. E., and EVANS, H. M.	Duality of Pituitary Gonadotrophins. Effects of FSH-ICSH Mixture on Hypophysectomized Male and Female Rats	723
FREEMAN, G.	Lack of Immunity to Latent Virus	568
GARDNER, W. U., and WHITE, A.	Mammary Growth in Hypophysectomized Male Mice Receiving Estrogen and Prolactin	590
GEIGER, A. J., MONROE, W. M., and GOODYER, A. V. N.	Clinical Fetal Electrocardiography. Its Practical Accomplishment	646
GLENN, F., 730.		
GOLDINGER., J. M., 570.		
GOODYER, A. V. N., 646.		
GRAVES, R. W., 555.		
GUTMAN, A. B., and GUTMAN, E. B.	A Phosphorylase in Calcifying Cartilage	687
GUTMAN, E. B., 687.		
GUZMAN BARRON, E. S., and GOLDINGER, J. M.	Effect of Iodoacetate and Malonate on Respiration of Sea Urchin Sperm	570
HAIG, C., and POST, J.	Vitamin A Concentration in Rat Liver During Recovery from CCl_4 Cirrhosis	710
HAMMON, W. M., and IZUMI, E. M.	Armstrong Mouse Adapted Poliomyelitis Virus: Effect of pH of Inoculum, and of "Enteric Organism Filtrate"	579
HANDLEY, C. A., SWEENEY, H. M., and BROOKMAN, B. T.	Metabolism in Perfused Dog's Head During Sodium Pentobarbital Depression and Metrazol Stimulation	670
HARDT, L. L., and STILL, E. U.	Thiamin in Sweat	704
HAYES, M., 728.		
HAYNES, F. W., 720.		
HEYS, F. M., 707.		
HOLT, L. E., JR., ALBANESE, A. A., BRUMBACK, J. E., JR., KAJDI, C., and WANGERIN, D. M.	Nitrogen Balance in Experimental Tryptophane Deficiency in Man	726
HOLT, L. E., JR., 728.		
HOOBLER, S. W., 720.		
HOUSE, V., 620.		
IZUMI, E. M., 579.		
JACOBS, J. L., KEILEY, J. J., and SOMMERS, S. C.	Hereditary Predisposition to Sensitization in Guinea Pigs	639
JAHN, T. L., and WULFF, V. J.	Influence of a Visual Diurnal Rhythm on Flicker Response Contours of Dytiscus	660
	Retinal Pigment Distribution in Relation to a Diurnal Rhythm in the Compound Eye of Dytiscus	656
KAJDI, C., 726, 728.		
KAUER, G. L., 730.		
KEILEY, J. J., 639.		
KEMPF, A. H., 631.		
KEMPF, J. E., 604.		
KLIGLER, I. J., 717.		
LALICH, J. J., 616.		
LAWRENCE, C. A., and SPRAGUE, K. D.	Effect of Sulfanilamide, Sulfapyridine, Sulfathiazole and Sulfanilylguanidine upon Colon-Typhoid-Dysentery Group	696
LEVIN, A. J., 624.		
LI, C. H., 723.		

LIBERSON, W. T., and STRAUSS, H. Electroencephalographic Studies: Slow Activity During Hyperventilation in Relation to Age 674
LIPSCHUTZ, A., and VARGAS, L., JR. Influence of Age on Serosal Tumoral Reaction of Guinea Pig Towards Estrogens 584
LYONS, W. R., SIMPSON, M. E., and EVANS, H. M. Influence of Lactogenic Preparations on Mammary Glands and Time of Vaginal Opening in Young Rats 634
McCOLLUM, E. V., 642.
McKENZIE, F. F., 648, 654.
McSWAIN, B., 730.
MACKENZIE, C. G., and McCOLLUM, E. V. Effect of Oral and Parenteral Administration of Vitamin E on Creatinuria and Symptoms of Dystrophic Rabbits 642
MAJOR, R. H., 616.
MARBURG, O., and SHWARTZMAN, G. Brain Reactions to Bacterial Filtrates Potent in Eliciting Local Tissue Reactivity 612
MERINO, C., DOAN, C. A., WOOLPERT, O. C., SCHWAB, J., and SASLAW, S. Reactions of Monkeys to Mixed Influenza Virus and Streptococcal Infections 563
MERINO, C., 558, 560, 566.
MONROE, W. M., 646.
MULLIGAN, R. M. Effects of Repeated Small Doses of Roentgen Rays on Canine Blood and Bone Marrow 607
MULLIKEN, B., 598.
NUNGESTER, W. J., and KEMPF, A. H. Recently Isolated Strains of Pneumococci as Incitants of Experimental Pneumonia in Rats 631
PIERCE, M. E., KEMPF, J. E., and SOULE, M. H. Effect of Dilution of Neutral Mixtures of Eastern Equine Encephalomyelitis Virus and Antiserum 604
PONDER, E. Acceleration of Hemolysis in Relation to Chemical Structure. III. The C-10 Alcohols 602
POST, J., 710.
RICHTER, C. P. Decreased Carbohydrate Appetite of Adrenalectomized Rats 577
RICHTER, C. P., and CLISBY, K. H. Graying of Hair Produced by Ingestion of Phenylthiocarbamide 684
ROBBINS, E. B., 676.
ROSEN, M. J., 575.
ROVENSTINE, E. A., 669.
SASLAW, S., 558, 560, 563, 566.
SCHALES, O., HOOBLER, S. W., and HAYNES, F. W. Cardiovascular Effects of Renin 720
SCHECHTMAN, A. M., and CANNON, J. A. An Experimental Study of Factors Inhibiting Differentiation of Proctodeum 628
SCHWAB, J., SASLAW, S., WOOLPERT, O. C., MERINO, C., and DOAN, C. A. Reactions of Monkeys to Experimental Respiratory Infections. Response to *Streptococcus hemolyticus* Group C 560
SCHWAB, J. L., 558, 563, 566.
SHWARTZMAN, G., 612.
SIMPSON, M. E., 634, 723.
SOMMER, H., 715.
SOMMERS, S. C., 639.
SOULE, M. H., 604.
SPRAGUE, K. D., 696.

STERN, K., and DANCEY, T. E.	Injection of Heavy Water into the Cerebro-Spinal Fluid Space	619
STILL, E. U., 704.		
STRAUSS, H., 674.		
SULKIN, N. M., 624.		
SWEENEY, H. M., 670.		
TAKACS, W. S., 637.		
TISCHER, L. A., 637.		
TOBIN, C. E.	Some Effects of Thyrotropic Hormone on Pregnant Rat	592
TOOMEY, J. A., TAKACS, W. S., and TISCHER, L. A.	Poliomyelitis Virus from Flies	637
TROESCHER-ELAM, E., and EVANS, H. M.	Inadequacy for Mice of a Synthetic Diet Supplemented with All Known Vitamin B Factors	549
TURNER, J. C., and MULLIKEN, B.	Rate of Disappearance of Subcutaneous Vegetable Oil in Normal and Castrate Mice	598
VARGAS, L., JR., 584.		
WANGERIN, D. M., 726, 728.		
WEBER, C. J., LALICH, J. J., and MAJOR, R. H.	Reduction of 2-(p-Nitrobenzenesulfonamido)-Pyridine to Sulfapyridine in the Animal Body	616
WELT, L. G.	Urinary Excretion of Trichina Antigen in Experimental Trichinosis	587
WHITE, A., 590.		
WINCHESTER, C. F., and McKENZIE, F. F.	Influence of Cell Concentration on Respiration Rate of Sperm	648
	Influence of Hydrogen Ion Concentration on Respiration Rate of Sperm	654
WOODHALL, B., 555.		
WOOLPERT, O. C., SCHWAB, J. L., SASLAW, S., MERINO, C., and DOAN, C. A.	Reactions of Monkeys to Experimental Respiratory Infections. Response to Influenza Virus A	558
WOOLPERT, O. C., 560, 563, 566.		
WULFF, V. J., 656, 660.		
YOUNGSTROM, K. A., WOODHALL, B., and GRAVES, R. W.	Acetylcholine Esterase Content of Brain Tumors	555
ZUBIN, J., and BARRERA, S. E.	Effect of Electric Convulsive Therapy on Memory	596

BOUND BY
LINNEMANN
BALTIMORE, MD